Assessing hemlock woolly adelgid induced decline and susceptibility using hyperspectral technologies

Jennifer Pontius

University of New Hampshire, Durham

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Assessing hemlock woolly adelgid induced decline and susceptibility using hyperspectral technologies

Abstract
The ultimate goal of this study was to provide the scientific framework for using narrow band hyperspectral instruments to assess early hemlock decline and susceptibility to the introduced hemlock woolly adelgid (HWA). To this end, spectral data from an ASD FieldSpec Pro was used to develop a 6-term linear regression equation, which predicted a detailed decline rating (0–10) with an R^2 of 0.71 and RMSE of 0.591. To scale up this method to a remote sensing platform, NASA's Airborne Visible Infrared Imaging Spectrometer (AVIRIS) was used to create a hemlock abundance map, correctly identifying hemlock dominated pixels (>40% basal area) with 88% accuracy. Reflectance at a chlorophyll sensitive wavelength (683nm), coupled with a water band index (R970/900), was able to predict decline with 85% accuracy. The extreme accuracy at the low (0–4) end of the range indicated that these wavelengths might be used to assess early decline, before visual symptoms are apparent.

Because instruments like AVIRIS have the capability to map foliar chemistry, the identification of links between HWA dynamics and foliar chemistry may be used to map relative susceptibility. To this end, we employed a three-tiered approach examining resistant vs. susceptible hemlock species, foliar chemistry vs. colonization success and regional foliar chemistry vs. HWA population levels. We found that HWA resistant hemlock species demonstrated higher concentrations of Ca and P, and lower concentrations of N and K. Regardless of host species, successful colonization of uninfested hemlocks was associated with higher N, and lower Ca and P concentrations. Regionally, higher concentrations of Ca, Mn, N and P were correlated with higher HWA densities. We hypothesize that higher N and K concentrations may have a palatability effect, driving HWA population levels, while higher concentrations of Ca and P may act as deterrents to more severe infestations.

These results indicate that by using hyperspectral remote sensing instruments, it is possible to identify the very early stages of hemlock decline and map relative susceptibility to HWA on a landscape scale. Such tools are instrumental in targeting management activities and ultimately controlling the HWA outbreak.

Keywords
Environmental Sciences, Agriculture, Forestry and Wildlife, Biology, Ecology, Remote Sensing

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ASSESSING HEMLOCK WOOLLY ADELGID INDUCED DECLINE AND SUSCEPTIBILITY USING HYPERSPECTRAL TECHNOLOGIES

BY

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DISSERTATION

Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of

Doctor of Philosophy
in
Earth and Environmental Science

September, 2004
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July 16, 2004
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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .................................................................................................................. III

LIST OF TABLES .......................................................................................................................... IX

LIST OF FIGURES ....................................................................................................................... XI

ABSTRACT ...................................................................................................................................... XVI

CHAPTER PAGE

INTRODUCTION ............................................................................................................................ 1

HWA Biology and History ........................................................................................................... 4

Forest and Ecosystem Response to HWA .................................................................................... 6

Hemlock Susceptibility to HWA .................................................................................................. 8

Near-Infrared Spectroscopy and Hemlock Decline ................................................................. 11

I. FOLIAR CHEMISTRY LINKED TO INFESTATION AND SUCCESS OF HEMLOCK WOOLY ADELGID .................................................................................................................. 14

Abstract ..................................................................................................................................... 14

Introduction ................................................................................................................................ 16
## Methods

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-Species Comparison</td>
<td>18</td>
</tr>
<tr>
<td>Colonization Study</td>
<td>18</td>
</tr>
<tr>
<td>Regional T. canadensis Study</td>
<td>19</td>
</tr>
<tr>
<td>Study Area</td>
<td>19</td>
</tr>
<tr>
<td>Foliage Sampling and Analysis</td>
<td>20</td>
</tr>
<tr>
<td>Infestation and Health</td>
<td>21</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>22</td>
</tr>
</tbody>
</table>

## Results

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-Species Comparison</td>
<td>24</td>
</tr>
<tr>
<td>Colonization Study</td>
<td>26</td>
</tr>
<tr>
<td>Regional Study</td>
<td>28</td>
</tr>
<tr>
<td>Stand Characteristics</td>
<td>28</td>
</tr>
<tr>
<td>Foliar Chemistry</td>
<td>31</td>
</tr>
<tr>
<td>Predictive Models</td>
<td>32</td>
</tr>
</tbody>
</table>

## Discussion

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species Comparison</td>
<td>36</td>
</tr>
<tr>
<td>Colonization Study</td>
<td>37</td>
</tr>
<tr>
<td>Regional Study</td>
<td>40</td>
</tr>
<tr>
<td>Predictive Models</td>
<td>44</td>
</tr>
</tbody>
</table>

## Conclusions

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
</tr>
</tbody>
</table>
II. ASSESSING HEMLOCK DECLINE USING VISIBLE AND NIR SPECTROSCOPY: SIGNATURE ANALYSIS, INDICES COMPARISON AND ALGORITHM DEVELOPMENT

Abstract ........................................................................................................................................ 46

Introduction .................................................................................................................................. 47

Methods ....................................................................................................................................... 49

Spectral Data Collection ........................................................................................................... 50

Full Spectrum Calibration ......................................................................................................... 51

Signature Analysis .................................................................................................................... 52

Statistical Analyses .................................................................................................................. 54

Results ....................................................................................................................................... 54

MPLS Regression ....................................................................................................................... 54

Indices ........................................................................................................................................ 59

Linear Regression ...................................................................................................................... 61

Discussion .................................................................................................................................. 62

Identification of Pre-Visual Decline Symptoms ........................................................................ 67

Conclusions ............................................................................................................................... 69
III. USING AVIRIS TO ASSESS HEMLOCK ABUNDANCE AND EARLY DECLINE IN THE CATSKILLS, NEW YORK ........................................................71

Abstract .............................................................................................................................71

Introduction .....................................................................................................................72

Methods .............................................................................................................................75

Remote Sensing Data .................................................................................................75

Hemlock Abundance ..................................................................................................77

Hemlock Decline .........................................................................................................78

Results and Discussion ...................................................................................................79

Hemlock Abundance ..................................................................................................79

Hemlock Decline ........................................................................................................83

Conclusions ......................................................................................................................90

FINAL CONCLUSIONS .....................................................................................................91

LITERATURE CITED ........................................................................................................93
LIST OF TABLES

Table 1. A summary of health characteristics associated with each decline category.
   The best-fit categories for each of the individual measurements were averaged to determine which decline rating best described the tree overall. 22

Table 2. A list of statistical analyses employed for the various components of this study. DF indicates the degrees of freedom available for error estimation after degrees are removed for model comparison. 24

Table 3. Spearman’s Rho Rank Correlations are reported for foliar chemistry analyzed before and after colonization of host trees, including correlations with the number of live sistens present after two generations and the number of old ovisacs present at the end of the study. 27

Table 4. A breakdown of the number of plots on which each species was found and the mean basal area (BA) for each species when present. 29

Table 5. A summary of measured variables on infested and uninfested plots. Significant t-tests (p< 0.01) are in bold and are based on DF = 44. 30

Table 6. A. Correlations based on the 225-sample regional data set highlight significant relationships (p < 0.01) between foliar chemistry, infestation and decline parameters. B. Partial correlations for only the significant pairwise variables are also included in order to quantify the relationships with infestation and decline independent of multi-collinearity effects. 31

Table 7. A list of existing indices included in our analyses that are known to have strong relationships with stress specific physiological responses (i.e. reductions in photosynthesis or chlorophyll content). 53
Table 8. Results from independent validation using various full spectrum and index regression models. The best results were obtained for the 5-term linear combined index equation indicating that a combination of key wavelengths and established indices can accurately predict a detailed health rating system. By rounding decline to the nearest integer, accuracy results are also presented within 1 and 2 classes for the ten class rating system.

Table 9. Key variables from the MPLS and linear regressions are defined, including pairwise correlations with decline, the decline class first significantly different from healthy samples and known absorbance features. Variables are listed by correlation strength.

Table 10. Of the 70 basal area prism points used to validate the MTMF for hemlock abundance, the vast majority were dominated by evergreen species. These points cover a range from 0 to 100 percent hemlock with an average of 49% hemlock basal area.

Table 11. AVIRIS variables significantly (p < 0.20) correlated with decline and known absorbance features. Variables are listed by correlation strength. Only R683nm and the WBI were retained for inclusion in the stepwise linear regression to predict decline.
LIST OF FIGURES

Figure 1. A schematic of the overall concept behind this study. The ultimate products include susceptibility indicators, decline and hemlock abundance maps for the Catskills region............................................................... 3

Figure 2. Counties with recorded sightings of Hemlock Woolly Adelgid infestation (USDA Forest Service, Forest Health Protection, Durham, NH). ..................... 5

Figure 3. An example of how the relative concentrations of an element associated with the production of defensive compounds (y axis, calcium) and an element that has a direct palatability effect (x axis, nitrogen) can work together to create varying degrees of host resistance to insect attack............ 10

Figure 4. The regional study area included 45 plots across six states, covering a range of infestation histories, site nutrient status and landscape characteristics. Circles represent study plot locations................................. 20

Figure 5. Hsu’s MCB means comparison highlight which species were significantly different from the susceptible eastern hemlock......................... 25

Figure 6. ANOVA’s comparing hemlock collected from within their native habitats showed that eastern hemlock had significantly higher N and lower P than the resistant western species (p < 0.001). Open points represent individual data and colored points indicate means and 95% confidence intervals for each species. Promising is the amount of overlap between western and some eastern hemlock trees.................................................. 26
Figure 7. Using only post infestation K and P concentrations, the number of live
sistens present at the end of the colonization study could be predicted
with an $R^2 = 0.53$ and RMSE = 46. This relationship held across all
species, with resistant and susceptible characteristics. ............................. 28

Figure 8. Linear regression models to predict HWA infestation levels and hemlock
decline exemplify how much of the variability can be explained using
only N, K, P and Ca.......................................................................................... 33

Figure 9. A stepwise linear regression model based only on landscape
characteristics was able to predict hemlock decline with an $R^2 = 0.37,$
RMSE = 0.90 and a 1-class tolerance accuracy of 90%................................. 34

Figure 10. The best-fit predictive model included both a mix of landscape and
chemistry variables. This 8-term model was able to predict decline with
an $R^2 = 0.68$, RMSE = 0.64 and 98% 1-class tolerance accuracy............... 35

Figure 11. ANOVA shows a significant difference between infested and uninfested
groups for nitrogen ($p = 0.0002$), calcium ($p = 0.0011$) and phosphorus
($p < 0.0001$). Cumulative frequency plots show that the highest
infestation levels (black) are typically associated with the highest
nitrogen and mid to lower calcium and phosphorus concentrations.......... 41

Figure 12. Samples were stacked to opacity, illuminated by an artificial light source
and scanned with a spectrometer from 12 different angles. A ratio
between average sample radiance and radiance from a reference panel
was used to calculate average reflectance for each sample..................... 51
Figure 13. An independent validation set was used to test the predictive abilities of the MPLS regression model. This resulted in an $R^2$ of 0.50 and RMSE of 0.82. Treated as a class variable, the MPLS regression predicted decline with a one-class tolerance accuracy of 89%.  

Figure 14. Regression coefficients from the MPLS regression of raw reflectance spectra. Higher (absolute value) coefficients signify wavelengths with more information related to decline.  

Figure 15. The fractional difference highlights those areas where declining samples differ from typical reflectance for healthy samples (class 1 and 2 as the baseline at 1.00). Reflectance at 680nm, 694nm, 760nm, 800nm, 950nm and 1922nm were instrumental in predicting decline.  

Figure 16. First derivative spectra of the red edge inflection point (left) and the 1388nm region (right) highlight wavelengths key in predicting hemlock decline.  

Figure 17. A linear regression based on CMS, DCI, NDVI, reflectance at 950 and 1922 nm and the first derivative at 1388nm was tested on an independent validation set. Decline rating was predicted with an $R^2 = 0.67$ and RMSE = 0.82. Converting this data to a class variable showed 96% one-class tolerance accuracy.  

Figure 18. A close look at the visible and NIR portions of the spectra highlight those wavelengths found to be significant in predicting hemlock decline. Wavelengths in gray were key to the MPLS regression, while wavelengths in black were used in the linear equation.
Figure 19. Average health measurements for each decline class shows the sharp decline in percent new growth and percent live crown in the early stages of decline. Defoliation, characterized by fine twig dieback and canopy transparancy, increases consistantly across the range. Defoliation reaches levels typically picked up by field and multi-spectra remote sensing instruments between 3.5 and 4.5. Bars represent 1 standard deviation.

Figure 20. The steps involved in processing the raw AVIRIS imagery and then creating the hemlock abundance and decline coverages over the Catskills.

Figure 21. Hemlock abundance was predicted with an $R^2$ of 0.65 and RMSE of 12. Most error was manifest as under predicting stands with significant understory hemlock (bottom right gray) and pine dominated stands predicted to have hemlock (upper left gray). Circles represent hemlock and mixed hemlock stands, open squares are dominated by other evergreen species. Hemlock-dominated stands were differentiated from non-hemlock stands with 83% accuracy.

Figure 22. A map of percent hemlock basal area highlights the high concentration of hemlock in lowland and riparian areas. Circles represent independent prism plot validation points.

Figure 23. The two-term linear regression equation, based on R683nm and the WBI, predicted hemlock decline with an $R^2$ of 0.88 and RMSE of 0.23.
Figure 24. Applied to all pixels with greater than 45 percent hemlock basal area, the decline prediction highlights more severe decline symptoms in the southeastern corner of the region where HWA has the longest history in hemlock stands. Other stressors are not excluded from this analysis. ........... 88
ABSTRACT

ASSESSING HEMLOCK WOOLLY ADELGID INDUCED DECLINE AND SUSCEPTIBILITY USING HYPERSPECTRAL TECHNOLOGIES

by

Jennifer Anne Pontius

University of New Hampshire, September, 2004

The ultimate goal of this study was to provide the scientific framework for using narrow band hyperspectral instruments to assess early hemlock decline and susceptibility to the introduced hemlock woolly adelgid (HWA). To this end, spectral data from an ASD FieldSpec Pro was used to develop a 6-term linear regression equation, which predicted a detailed decline rating (0-10) with an $R^2$ of 0.71 and RMSE of 0.591. To scale up this method to a remote sensing platform, NASA’s Airborne Visible Infrared Imaging Spectrometer (AVIRIS) was used to create a hemlock abundance map, correctly identifying hemlock dominated pixels (>40% basal area) with 88% accuracy. Reflectance at a chlorophyll sensitive wavelength (683nm), coupled with a water band index (R970/900), was able to predict decline with 85% accuracy. The extreme accuracy at the low (0-4) end of the range indicated that these wavelengths might be used to assess early decline, before visual symptoms are apparent.

Because instruments like AVIRIS have the capability to map foliar chemistry, the identification of links between HWA dynamics and foliar chemistry may be used to map
relative susceptibility. To this end, we employed a three-tiered approach examining resistant vs. susceptible hemlock species, foliar chemistry vs. colonization success and regional foliar chemistry vs. HWA population levels. We found that HWA resistant hemlock species demonstrated higher concentrations of Ca and P, and lower concentrations of N and K. Regardless of host species, successful colonization of uninfested hemlocks was associated with higher N, and lower Ca and P concentrations. Regionally, higher concentrations of Ca, Mn, N and P were correlated with higher HWA densities. We hypothesize that higher N and K concentrations may have a palatability effect, driving HWA population levels, while higher concentrations of Ca and P may act as deterrents to more severe infestations.

These results indicate that by using hyperspectral remote sensing instruments, it is possible to identify the very early stages of hemlock decline and map relative susceptibility to HWA on a landscape scale. Such tools are instrumental in targeting management activities and ultimately controlling the HWA outbreak.
INTRODUCTION

Throughout history, invasions of plant pathogens have led to some of the most serious disruptions of natural ecosystems ever recorded. Due to increased technology and a global economy, the rate of exotic pest introduction has steadily increased (Liebhold et al. 1995). These biological invasions are second only to habitat loss in native species declines (Everett 2000).

The hemlock woolly adelgid (HWA), an invasive insect pest native to Japan, has caused widespread decline and mortality in hemlock forests across the eastern U.S. since the mid-1980's, and it appears that the pattern of mortality is likely to continue. The resulting effects on eastern forest ecosystem function and aesthetic value could be devastating.

Most assessments of decline following HWA infestation involve time-consuming field-based methods. Although these methods are valuable in monitoring gross changes over time, they are not able to identify trees in the very early stages of decline (Sampson et al. 2000) or assess large tracts on a regional scale. By the time a tree displays visual symptoms of decline, it can already be adversely affected. The ability to identify trees in the very early stages of decline across large forest tracts will help direct management activities, with hopes of ultimately controlling HWA outbreaks.

A mounting body of evidence suggests that leaf optical properties can be used to identify stress condition in trees, particularly narrow-band hyperspectral sensors (Carter 1993; Carter and Miller 1994; Zarco-Tejada et al. 2000b). The purpose of this study was
to determine if narrow-band hyperspectral data at the tree (benchtop) and plot (remote sensing) level are sufficient to predict a detailed hemlock decline rating system, including pre-visual decline symptoms.

Most infested eastern hemlock (*Tsuga canadensis* (Carriere)) have shown no resistance to HWA and little chance for recovery (McClure 1995b). However, there is evidence that the impact of HWA varies significantly with site conditions and the presence of other stressors, with adjacent stands often responding differently to attack (Orwig and Foster 1998; Sivaramakrishnan and Berlyn 1999).

To date, no one has addressed how foliar nutrient status relates to HWA infestation and hemlock decline. In order to fully understand these relationships, this study took a multi-tiered approach: inter-species comparisons, colonization and regional studies. It is only through the confluence of evidence from such multiple “mini” studies that a strong case for the role of foliar chemistry may be made. Because hyperspectral sensors are able to map foliar canopy chemistry, establishing the link between foliar chemistry and HWA dynamics / hemlock decline will allow land managers to map susceptibility on a landscape scale.

Because HWA infestation is ongoing, this study is a “test case” of the abiotic controls on an introduced exotic pest and the early response of trees to stress resulting from infestation. The research proposed here will lay the foundation for a comprehensive and publicly accessible, regional-scale HWA prediction and monitoring effort, and could also inform monitoring efforts for other invasive species. Specifically, we will (Figure 1):
I. Quantify the role of foliar chemistry in HWA population dynamics and subsequent hemlock decline.

II. Identify narrow-band wavelengths and indices to predict a detailed hemlock decline rating at the tree level, using a benchtop NIR spectrometer.

III. Adapt the techniques developed at the tree level in II to a remote sensing platform to assess plot level hemlock decline on a landscape scale.

Figure 1. A schematic of the overall concept behind this study. The ultimate products include susceptibility indicators, decline and hemlock abundance maps for the Catskills region.

These techniques provide a much-needed tool for the early detection of stressors such as HWA infestation, and will allow forest land management agencies to focus biological control efforts on incipient infestations before trees are severely impacted. In conjunction with existing methods for mapping foliar chemistry (Martin and Aber 1997; Hallett et al. 1997; Smith et al. 2002), development of this capability could greatly
enhance our ability to detect patterns of hemlock susceptibility, adelgid infestation limits and hemlock decline across large spatial scales.

Biological invasions can alter population dynamics, community structure, disturbance regimes and ecosystem level processes (Mack and D'Antonio 1998; Von Borembsen 1989; Liebhold et al. 1995; Jenkins et al. 1999; Orwig and Foster 1998; Yorks et al. 2000). Invasions by chestnut blight and Dutch elm disease are examples that have caused catastrophic tree mortality and the virtual elimination of previously dominant tree species.

Eastern hemlock forests are currently faced with invasion by the hemlock woolly adelgid (HWA). It is predicted that infestation will lead to unprecedented hemlock loss, regardless of stand age, size, stocking, site conditions, or location (Orwig et al. 2002). Abrupt overstory removal of hemlock could generate a lengthy and dramatic period of forest reorganization leading to completely new forest types, changes in wildlife assemblages and profound ecosystem impacts (Orwig et al. 2002).

**HWA Biology and History**

The HWA is thought to be a native of Japan where it is a common, but innocuous inhabitant of hemlock and spruce forests (McClure 1995a; McClure M.S. et al. 1996). In the early 1920’s, accidental introductions from Asia first brought it to western North America, where western hemlock species (*Tsuga mertensiana* Bong. Carr.) and *Tsuga heterophylla* Raf.) also appeared to be resistant. Some thirty years later, HWA made its first appearance on the east coast in Richmond, Virginia (Souto et al. 1995). Dispersed by birds, deer, wind and humans (McClure 1990), it spread to the Connecticut coastline where resulting hemlock declines were first documented in 1985 (McClure 1987). HWA
now occurs in parts of eleven states along the eastern seaboard from North Carolina to Maine (Figure 2). Current rates of spread are estimated at 10-15 miles per year into uninfested areas with all indications that it will be able to penetrate hemlock's entire range (Orwig and Foster 1998).

Hemlock Woolly Adelgid Infestations 2003

Hemlock Woolly Adelgid infestations

- Infested Counties
- Counties Infested in 2003
- Uninfested Counties

Native Range of Hemlock

Figure 2. Counties with recorded sightings of Hemlock Woolly Adelgid infestation (USDA Forest Service, Forest Health Protection, Durham, NH).

HWA is a “sucking” forest pest that attaches to its host on new growth at the base of the needles. A stylet bundle is inserted near the juncture with the stem where it penetrates deep within the plant tissue to the parenchyma cells of the xylem rays. Here HWA depletes the needle of its stored nutrient supplies and photosynthate (Shields et al. 1995; Shields et al. 1995). Saliva is secreted creating protective tracks and sheaths for
the stylet bundles (McClure 1995a; Shields et al. 1995). The mechanisms of HWA damage thus include both depleting the needle’s supply of nutrients and photosynthate and injecting toxic saliva. In the balsam woolly adelgid (Fowler et al. 2001), this saliva can trigger an imbalance in plant hormones, causing a modification of the xylem and restriction of water uptake by the sapwood (Hain 1988). Infestation leads to needle desiccation, discoloration and ultimately, needle loss (McClure M.S. et al. 1996). New growth is stunted (Ward et al. 1992) and dieback of limbs and entire branches usually follows within several years (McClure M.S. et al. 1996).

HWA population densities fluctuate annually, mainly in response to density dependent changes in the nutritional quality of hemlock (McClure 1991). Although populations seem to be negatively affected by severe winter cold, recovery has been rapid (Souto et al. 1995). Lower winter temperatures in northern New England may retard its spread, but the adelgid will probably develop sufficient cold-hardiness to expand its distribution through the entire range of hemlock (McClure 1995a). Unless novel management techniques can be developed, lack of host resistance could lead to the eradication of eastern hemlock in all but small patches across its entire range (Foster 1999).

**Forest and Ecosystem Response to HWA**

Once HWA becomes established on hemlock, dramatic decline in new growth production is the first symptom (Ward et al. 1992). Flushes of new growth then inversely cycle with HWA populations for several years in a density-dependent feedback loop (McClure M.S. et al. 1996). As nutrient reserves are depleted, defoliation and chlorosis progresses, followed by fine twig and branch dieback. Complete mortality typically
occurs within 5-6 years of the initial infestation, although some trees have been known to survive for more than 10 years (McClure M.S. et al. 1996; Orwig 2002). Generally, the stands with the highest mortality are those that have had a population of HWA for the longest time (Mayer et al. 1998). However, exceptions to this pattern may occur, depending on environmental conditions (Mayer et al. 1998) and water stress (Onken 1995).

Most researchers now agree that all hemlock may ultimately be susceptible to HWA (Orwig and Foster 1998; Foster 1999). Large canopy gaps, and in homogeneous stands, entire canopies may be opened. This is expected to reduce plant nutrient and water uptake, increase light attenuation, soil temperatures, decomposition and nutrient cycling rates (Jenkins et al. 1999; Yorks 2002).

Hemlock mortality can have dramatic effects on nutrient cycling rates. Mortality may result in nutrient leaching from the soil and nutrient loading to stream water (Jenkins et al. 1999). The resulting elevated nutrient leaching could lead to site nutrient capital reduction and in turn, a reduction in forest productivity (Yorks et al. 1999). Because healthy hemlock stands filter rainwater, prevent soil erosion, limit light availability and algal productivity, and have a large effect on soil nutrient cycling, the loss of hemlock in the overstory is likely to be associated with a decline in surface water quality (Yorks et al. 1999). The impact of hemlock mortality on surface water quality is likely to be exacerbated by the tendency of eastern hemlock to grow along streambeds and in ravines (Whitney 1990). This could impact the management of watersheds providing municipal water supplies.
In southern New England, HWA has initiated a rapid shift from a coniferous to a deciduous forest, with black birch (*Betula lenta* L.), red maple (*Acer rubrum* L.) and various oaks (*Quercus spp.*) growing up beneath the dying hemlock canopy (Orwig and Foster 1998). Such a dramatic change in forest species composition could be detrimental for the hundreds of avian, mammal, aquatic, amphibious and understory plant species that depend on the unique micro-habitats created within hemlock stands (Evans et al. 1995).

**Hemlock Susceptibility to HWA**

Many researchers predict that all hemlock in eastern North America are susceptible to HWA and that all hemlock stands in the region will eventually succumb (Foster 1999; Orwig and Foster 1998). However, there is evidence that even after many years of infestation, some sites show no adverse affects from substantial HWA populations (Bonneau et al. 1997; Souto et al. 1995). These differences make it nearly impossible to predict where or how much hemlock mortality will occur. A more complete understanding of the effects of the abiotic factors on HWA infestation and tree decline is essential in the development of a successful integrated pest management program (Gray and Salom 1995).

Water availability appears to play a major role in HWA population growth and hemlock decline. Sucking insects do not get as much nourishment when living on plants with an abundant water supply (Mayer et al. 1998) and hemlock are better able to respond to stress and recover when water levels are adequate (Onken 1995). In New Jersey, Mayer et al. (1998) found that the highest levels of hemlock mortality occurred on xeric sites and ridge tops where hemlock was growing under marginal conditions.
To date, very little research has been conducted to determine the effect of nutrient status and site characteristics on HWA infestation patterns and hemlock decline symptoms (McClure 1980). It is understood that herbivory is usually positively correlated with foliar nitrogen concentrations and that low nitrogen concentrations in foliage can limit insect populations (McClure 1980; Schowalter et al. 1986; White 1984). We also know that foliar chemistry often reflects the chemistry of the soils on which trees grow. It then follows that both soil and foliar chemistry should play a large role in HWA infestation patterns.

Host nutrition can modify a plant’s response to insects and its susceptibility to infestation by creating an environment more or less favorable to insect attack (Dale1988). This can be realized through a direct “palatability effect” on the concentrations of nutrients available in the foliage for insect consumption or an indirect effect of altered levels of secondary defense metabolites. Host nutrition studies focused on low host foliar nitrogen concentrations have found reduced growth rate, lower efficiency of conversion of ingested food, decreased fecundity and survival, as well as lower population levels for several insect herbivore species (Estiarte et al. 1994; Dale1988; Mattson 1980). High nitrogen levels also increase leaf palatability by increasing tissue softness and water content as carbohydrates are diverted for protein synthesis rather than the construction of cell walls (Tinsdale and Nelson 1975).

Resistance is also imparted through the production of defensive compounds, which may deter herbivory by reducing palatability or have direct toxic effects on insect populations (Janzen1979; Feeny 1976; Erlich and Raven 1965). The production of secondary compounds has also been linked to host nutrient supply (Gershenzon1983;
Kleiner et al. 1992; Estiarte et al. 1994). Palatability effects and defensive compounds often work together in plants imparting various degrees of resistance to the host species (Figure 3).

![Figure 3](image)

**Figure 3.** An example of how the relative concentrations of an element associated with the production of defensive compounds (y axis, calcium) and an element that has a direct palatability effect (x axis, nitrogen) can work together to create varying degrees of host resistance to insect attack.

Because they remain attached at one feeding site, sapsuckers appear particularly sensitive to host quality as opposed to quantity of available forage. The performance of several species of aphids and plant hoppers has been directly linked to leaf photosynthate levels (Dale1988). Studies also show that aphids may be particularly sensitive to amino nitrogen levels in the phloem sap and turgor pressure of the host plant (Dale1988; Schowalter et al. 1986).
The only “nutrient status” studies directly addressing HWA have focused primarily on the effect of nitrogen fertilizer amendments to infected hemlock stands or insect response to subtle changes in the nitrogen concentrations in their host plant. McClure (McClure 1991; McClure 1991) found that each of the piercing and sucking insects that affect hemlock showed significant increases in survival, fecundity and developmental rate as a result of nitrogen fertilization. This led to increased hemlock decline even after only a single HWA generation (McClure 1991). To date, no attention has been paid to the role of other essential macronutrient foliar concentrations in insect population patterns.

Near-Infrared Spectroscopy and Hemlock Decline

Two recent advances have been made in hyperspectral remote sensing relative to the objectives of this proposal: 1) an improved ability to identify species through their visible and infrared reflectance characteristics and 2) the ability to use this same spectral data to determine chemical and physiological characteristics of foliage in the forest canopy.

Hyperspectral imaging spectrometers differ from the more commonly used remote sensing instruments in that they measure many contiguous narrow spectral bands which increases functionality by allowing different analytical techniques based on absorption features in the observed materials. The hyperspectral techniques used in this study have been developed from an airborne instrument, the Airborne Visible/Infrared Imaging Spectrometer (Green et al. 1998), measuring 224 contiguous bands in the range of 0.4μm – 2.5 μm. The spatial resolution of AVIRIS varies from 4-5m to 18-20m when flown at 12,500ft and 60,000ft, respectively.
AVIRIS derived maps of foliar chemistry have been used to estimate forest productivity, soil characteristics and nutrient cycling on a landscape scale based on extensive field studies relating foliar chemistry to these ecosystem processes (Smith et al. 2002; Ollinger et al. 2000). These same techniques could be used to map relative susceptibility to HWA based on the relationship between foliar chemistry and HWA dynamics.

Using these techniques, hemlock stands were accurately classified at the Harvard Forest in Central Massachusetts (Martin et al. 1998). The resulting AVIRIS species maps generated in this study showed greater spatial detail than maps based solely on field sampling, with field validation demonstrating that the maps derived from hyperspectral data more closely matched field conditions than field survey maps. This can be attributed to the fact that AVIRIS samples every point on the ground in contrast to the relatively sparse sampling of typical field surveys.

External stress factors can induce changes in leaf structure and function, which in turn modify the characteristics of light reflected from the leaf (Rock et al. 1986; Tucker 1980). Even subtle changes can alter reflectance across the visible and NIR spectrum (Lichtenthaler 1996).

There is mounting evidence that hyperspectral instruments have the capability to not only assess defoliation, but also identify the early signs of stress, in some cases before visual symptoms are apparent (Cibula and Carter 1992; Mohammed et al. 1995; Zarco-Tejada et al. 2000a; Zarco-Tejada et al. 2000c). This can be explained by the tendency of stressed leaves to experience reduction in photosynthetic activity and to lose chlorophyll. These changes alter reflectance at chlorophyll sensitive wavelengths (Carter and Knapp...
Chlorophyll$_a$ and \( \delta \) content are particularly good detectors of stress because of their direct role in photosynthesis. Narrow wavebands near 700nm where changes in chlorophyll absorption are easily detectable have been recommended for early detection of forest damage (Hoque et al. 1990; Hoque et al. 1992) and were able to detect decreased vigor, before visual symptoms were apparent, in pine seedling canopies (Cibula and Carter 1992). Because changes in chlorophyll function typically precede changes in chlorophyll content, chlorophyll fluorescence has also been shown to be a useful tool in identifying pre-visual strain (Zarco-Tejada et al. 2000a; Zarco-Tejada et al. 2000c).

Such diagnostic features for estimating plant decline are located in relatively narrow wavebands, interspersed with insensitive features (Treitz and Howarth 1999). Ratios or pairs of wavelengths (indices) tend to highlight significant features while correcting for geometrical and background effects (Baret and Guyot 1991). This is accomplished by targeting stress sensitive bands, while including an insensitive "control" band which functions as a baseline to exclude variability due to other factors (Treitz and Howarth 1999). Such simple transformations have been closely correlated with plant characteristics, without the sensitivity to external variables such as sun angle or instrument variability (Pinty et al. 1993).
CHAPTER I

FOLIAR CHEMISTRY LINKED TO INFESTATION AND SUCCESS OF HEMLOCK WOOLY ADELGID

Abstract

The hemlock woolly adelgid (HWA) is an invasive insect pest that is predicted to lead to widespread mortality of hemlock across its native range. The highly variable impact of HWA in the field has been primarily attributed to site or climatic variables. The objective of this study was to determine if foliar chemistry could be linked to HWA success and subsequent hemlock decline using a three-tiered approach: 1) comparison of resistant vs. susceptible species, 2) examination of foliar chemistry and colonization success, and 3) a regional eastern hemlock study to investigate relationships between foliar chemistry and HWA/hemlock health across its native range. HWA resistant species demonstrated higher concentrations of Ca and P, and lower concentrations of N and K. Regardless of host species, successful colonization of uninfested hemlocks was associated with higher N, and lower Ca and P concentrations. Across the Northeast, higher concentrations of Ca, Mn, N and P were correlated with higher HWA densities. Based on the results of this research, we hypothesize that higher N and K concentrations may have a palatability effect, driving HWA population levels, while higher concentrations of Ca and P may be a defensive response, acting as a deterrent to more...
severe infestations. Using foliar chemistry alone, over half of the variability in hemlock decline could be accounted for. These results indicate that foliar chemistry may be used to assess relative susceptibility to HWA across the Northeast and should be included in susceptibility models.
Introduction

Since the 1980's when the hemlock woolly adelgid (HWA, *Adelges tsugae*, Annand (Homoptera: Adelgidae) was introduced to the northeastern U.S., it has spread rapidly, leading to eastern hemlock (*Tsuga canadensis* Carriere) decline and mortality from North Carolina to Massachusetts (Souto et al. 1995). Most infested hemlock have shown no resistance to HWA and little chance for recovery (McClure 1995b). However, there is evidence that the impact of HWA varies significantly with site conditions and the presence of other stressors, with adjacent stands often responding differently to attack (Orwig and Foster 1998; Sivaramakrishnan and Berlyn 1999).

Among hemlock species, *Tsuga diversifolia* (Masters), *T. chinensis* (Franch.) and *T. sieboldii* (Carriere) have shown high tolerance to HWA infestation, with minimal impact to hemlock populations. This has been attributed to a combination of natural predators and host resistance (McClure 1995b). However, in the western U.S. where natural predators are non-existent, *T. heterophylla* (Sargent) and *T. mertensiana* (Carriere) are also resistant to HWA (McClure 1992). The mechanisms behind this host resistance remain unclear.

Studies of *T. canadensis* have examined the existence of predisposing factors that may speed decline. While most of these identify site factors directly or indirectly related to water availability (Bonneau et al. 1997; Onken 1995; Royle and Lathrop 1999), Royle and Lathrop (Royle and Lathrop 1999) suggest that other factors must be involved in order to more fully explain the variability witnessed in the field.

We hypothesize that a relationship exists between foliar nutrient status, HWA infestation and hemlock decline. For example, it is understood that herbivory is usually
positively correlated with foliar nitrogen concentrations and that low nitrogen concentrations in foliage can limit insect populations (McClure 1980; Schowalter et al. 1986; White 1984). However, no attention has been paid to the role of other essential macronutrient foliar concentrations in HWA population patterns. Of particular interest are Ca and P, which have been implicated in suppressing aphid populations on agricultural crops (Chhillar and Verma 1985; Harada et al. 1996).

In order to fully understand the relationship between foliar chemistry, HWA and hemlock decline, this study took a multi-tiered approach, with inter-species comparisons, colonization and regional studies. The specific objectives were:

- Determine if foliar element concentrations in HWA resistant hemlock species are different from eastern hemlock (Inter-species Comparison).
- Determine whether foliar chemistry is related to colonization success on a suite of hemlock species (Colonization Study).
- Examine relationships among foliar element concentrations, HWA population density and severity of post infestation eastern hemlock decline on a regional scale (Regional T. canadensis Study).

If there are significant differences in foliar chemistry between resistant and susceptible species, and these same elements are strongly correlated with colonization success, then we hypothesize that such elements may play a role in host susceptibility. If these same relationships hold within eastern hemlock across our regional study, then foliar chemistry may also help identify stands relatively less susceptible to HWA.
Methods

Inter-Species Comparison

Foliage was collected from four HWA resistant species *T. diversifolia* (TSDI)(n=4), *T. sieboldii* (TSSI)(n=2), *T. heterophylla* (TSHE)(n=4), *T. chinensis* (TSCH) (n=2) and the non-resistant *T. canadensis* (TSCA)(n=5) growing at the Arnold Arboretum in Jamaica Plain, MA in June 2001. All trees were mature, landscape specimens, growing on similar soil type and under similar light, climate and growth conditions. Hand shears were used to collect terminal branches from multiple sides of each study tree.

Foliar lignin, N and P data were also obtained for fifty *T. heterophylla* (TSHE) and five *T. mertensiana* (TSME) collected in the Pacific Northwest and archived at Oak Ridge National Laboratory Distributed Active Archive Center (Matson 1994) for comparison to 44 eastern hemlock samples from the NERC database (NERC 2003).

Colonization Study

The Arnold Arboretum trees described above were artificially infested by attaching multiple, heavily HWA infested *T. canadensis* branches to branches on each study tree. They were then contained with mesh bags. After two HWA generations (6 months), bagged branches were collected and the number of live sistens and old ovisacs on the recipient host branch was recorded. Foliar chemistry was determined for pre-infestation (collected coincident with attachment of HWA infested branches) and post-infestation (bagged samples collected at the end of the colonization study) using the methods described below.
Regional T. canadensis Study

Study Area

In 2001, 45 HWA monitoring plots (20m x 20m) were established with the assistance of federal, state and private land managers at locations where hemlock was the dominant species. These were selected to represent the range of infestation levels, infestation histories, hemlock decline symptoms and site nutrient status found across the northeastern U.S. Plots were located in Connecticut, Massachusetts, New Hampshire, New Jersey, New York and Pennsylvania (Figure 4). Within each plot, 5 dominant or co-dominant hemlock trees were selected for foliage sampling and decline assessments. Diameter at breast height and species was recorded for all trees with >5 cm dbh within each plot.
Figure 4. The regional study area included 45 plots across six states, covering a range of infestation histories, site nutrient status and landscape characteristics. Circles represent study plot locations.

Foliage Sampling and Analysis

Foliage was collected from the mid and upper canopies of each tree using a 12-gauge shotgun. Foliage was placed in a plastic bag and refrigerated for a maximum of 72 hours until new growth and infestation levels could be assessed (see Infestation and Health below). Needles were dried at 70 °C and ground to pass a 1mm mesh screen. A NIRSystems spectrophotometer was used to measure N, lignin and cellulose concentrations (Bolster et al. 1996). Dried and ground foliage was digested using a
microwave assisted acid digestion procedure (EPA Method 3052 1996) and analyzed for Ca, K, Mg, Mn and P using a Varian axial inductively coupled plasma spectrometer.

**Infestation and Health**

For each sample hemlock tree, between 100 and 150 terminal branchlets were inspected for the presence of early instar HWA using a 10X hand lens. Infestation levels are reported as a percentage of terminals infested.

Hemlock decline was assessed using methods specifically designed to quantify the various, sequential symptoms of hemlock decline that follow adelgid infestation. This included the percent of terminal branchlets with new growth (R. Evans pers. comm.), percent transparency (quantified using a concave spherical densiometer (Pontius et al. 2002)), percent fine twig dieback and live crown ratio (USDA Forest Service 1997). The categories of hemlock health described in Table 1 reflect the typical characteristics for each health measurement at various stages of hemlock decline. Health category assignments for each measurement on a tree were averaged to determine one overall decline rating that best described the trees overall health (where 0 = perfect health, 10 = dead).
<table>
<thead>
<tr>
<th>Decline Class</th>
<th>Health Status</th>
<th>Typical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Perfect Health</td>
<td>All branchlets produce a flush of new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minimal canopy transparency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 90% of the total tree height is photosynthetically active</td>
</tr>
<tr>
<td>1</td>
<td>Very Healthy</td>
<td>Almost all branchlets produce new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Only 6 to 9% of the canopy is transparent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fine twig dieback is minimal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80 to 89% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>2</td>
<td>Healthy (typical healthy forest co-dominant)</td>
<td>Over 85% of terminal branchlets produce new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 to 14% canopy transparency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than 5% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70 to 79% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>3</td>
<td>Earliest Decline</td>
<td>Slight reductions in new growth production (80 to 84%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15 to 19% canopy transparency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 to 10% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65 to 69% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>4</td>
<td>Light Decline</td>
<td>More noticeable reductions in new growth (75 to 79%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Approaching 10% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60 to 64% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>5</td>
<td>Light to Moderate Decline</td>
<td>Less than 3/4 of branchlets are producing new growth (70 to 74%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 to 29% canopy transparency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 to 15% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 to 59% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>6</td>
<td>Moderate Decline</td>
<td>Only 60 to 69% of terminals produce a flush of new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 to 34% canopy transparency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Up to 15% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 to 49% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>7</td>
<td>Moderate to Severe Decline</td>
<td>Barely half (40 to 59%) of terminals produce a flush of new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 to 39% of the canopy is transparent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 to 39% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>8</td>
<td>Severe Decline</td>
<td>Barely 1/3 (20 to 39%) of terminals produce a flush of new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 to 44% of the canopy is transparent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Up to 20% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 to 29% of canopy is photosynthetically active</td>
</tr>
<tr>
<td>9</td>
<td>Near Death</td>
<td>Less than 20% of terminals produce a flush of new growth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>More than 45% of the canopy is transparent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Greater than 25% fine twig dieback</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than 20% of the canopy is photosynthetically active</td>
</tr>
<tr>
<td>10</td>
<td>Dead</td>
<td>100% defoliated</td>
</tr>
</tbody>
</table>

Table 1. A summary of health characteristics associated with each decline category. The best-fit categories for each of the individual measurements were averaged to determine which decline rating best described the tree overall.

**Statistical Analysis**

Pairwise multiple correlations were used to examine relationships between hemlock decline ratings, live sisten counts and foliar concentrations (Zar 1984). Due to the non-normal distribution of the percent infestation data, Spearman’s Rho rank
significance tests were used for correlation analyses (Pagano and Gauvreau 2000). Because many of the chemical variables included in this study were highly correlated with each other, partial correlations based on significant pairwise correlated variables were included in order to isolate relationships with infestation and decline with the effects of multi-collinearity removed (Selvin 1995). Because of limited sample size in the species and colonization studies, significance cut-off limits were raised to 0.10 to avoid type II errors.

To determine how much variability could be accounted for, multiple stepwise linear regressions were constructed for infestation and decline data in the regional study including site variables only, chemistry only and full combined models (Pagano and Gauvreau 2000). Variables were retained in the model if \( p < 0.01 \) and the variance inflation factor was below 2.0. Potential models were compared based on Mallow’s Cp and PRESS statistics (Kleinbaum et al. 1998).

All analyses were conducted using JMPin version 4.0.3 (Academic). Significance \( \alpha \)-cutoffs of 0.10, 0.10 and 0.01 were the nominal indicators of statistical significance for the interspecies comparison, colonization study and regional study respectively. A summary of statistical techniques can be found in Table 2.
Table 2. A list of statistical analyses employed for the various components of this study. DF indicates the degrees of freedom available for error estimation after degrees are removed for model comparison.

**Results**

**Inter-Species Comparison**

Comparisons of new growth foliage from various species at the Arnold Arboretum indicated that Eastern hemlock had significantly lower Ca and higher N, K and P than various resistant species (Figure 5).
Figure 5. Hsu’s MCB means comparison highlight which species were significantly different from the susceptible eastern hemlock (* indicates p < 0.10).
Comparisons of the western and eastern North American species collected within their native habitats showed that the resistant western species had significantly lower N and higher P (p < 0.0001) than Eastern hemlock (Figure 6). Hsu's MCB means comparison indicated that while TSHE and TSME were similar, both were significantly different than TSCA (p < 0.01).

![Figure 6. ANOVA's comparing hemlock collected from within their native habitats showed that eastern hemlock had significantly higher N and lower P than the resistant western species (p < 0.001). Open points represent individual data and colored points indicate means and 95% confidence intervals for each species. Promising is the amount of overlap between western and some eastern hemlock trees.](image)

**Colonization Study**

Of the 18 trees selected for the colonization study, 9 were successfully infested. The number of live sistens present after two HWA generations was positively correlated
with initial K concentrations and negatively correlated with post-infestation P concentrations using Spearman’s Rho Rank correlations ($p < 0.10$) (Table 3).

<table>
<thead>
<tr>
<th>Colonization Data</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>N</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Live Sistens Present</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Infestation Chemistry</td>
<td>-0.33 (0.19)</td>
<td>0.07 (0.80)</td>
<td>0.07 (0.78)</td>
<td>0.09 (0.74)</td>
<td>0.14 (0.57)</td>
<td>0.08 (0.76)</td>
</tr>
<tr>
<td>Post-Infestation Chemistry</td>
<td>0.03 (0.93)</td>
<td><strong>0.49 (0.07)</strong></td>
<td>-0.17 (0.55)</td>
<td>0.38 (0.16)</td>
<td>0.00 (0.99)</td>
<td><strong>-0.54 (0.04)</strong></td>
</tr>
<tr>
<td><strong>Number of Old Ovisacs Present</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Infestation Chemistry</td>
<td>-0.05 (0.86)</td>
<td>0.29 (0.25)</td>
<td><strong>0.43 (0.08)</strong></td>
<td>-0.12 (0.64)</td>
<td>0.33 (0.18)</td>
<td>0.20 (0.44)</td>
</tr>
<tr>
<td>Post-Infestation Chemistry</td>
<td>0.17 (0.55)</td>
<td><strong>0.45 (0.10)</strong></td>
<td>0.34 (0.22)</td>
<td>0.34 (0.22)</td>
<td>0.32 (0.25)</td>
<td>-0.18 (0.52)</td>
</tr>
</tbody>
</table>

Values represent Spearman’s Rho Rank Correlations (with $p$-values in parentheses). Bold highlights those variables significant at the 0.10 level.

Table 3. Spearman’s Rho Rank Correlations are reported for foliar chemistry analyzed before and after colonization of host trees, including correlations with the number of live sistens present after two generations and the number of old ovisacs present at the end of the study.

The number of old ovisacs present on host species was positively correlated with initial Mg and post-infestation K concentrations. Initial N concentrations were also weakly correlated with the number of ovisacs present (Table 3). Although not significant, there was a weak positive correlation between foliar N and the presence of old ovisacs. ANOVA’s indicated that colonization success was not significantly different between host species ($p = 0.39$) or assumed resistance group ($p = 0.97$). Using only the strongest correlates (K and P), we were able to predict the number of live sistens present with $R^2 = 0.53$ and RMSE = 46 (Figure 7).
Figure 7. Using only post infestation K and P concentrations, the number of live sizzleen present at the end of the colonization study could be predicted with an $R^2 = 0.53$ and RMSE $= 46$. This relationship held across all species, with resistant and susceptible characteristics.

Regional Study

Stand Characteristics

Across all plots, hemlock ranged from 45 to 95% of the total basal area; this was similar between infested and uninfested stands (Table 5). Selected plots were homogeneous, mature stands with a mean hemlock basal area of 21 m$^2$ha$^{-1}$ and 23 m$^2$ha$^{-1}$ for uninfested and infested stands respectively. In addition to eastern hemlock, the most commonly occurring species included red maple (*Acer rubrum* L.), yellow birch (*Betula alleghaniensis* Britt.), black birch (*Betula lenta* L.) and American beech (*Fagus grandifolia* Ehrh.) (Table 4).
<table>
<thead>
<tr>
<th>Species</th>
<th>No. Plots</th>
<th>Mean % BA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Hemlock</td>
<td>45</td>
<td>72</td>
</tr>
<tr>
<td>Red Maple</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>Yellow Birch</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>Black Birch</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>American Beech</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Black Oak</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>White Pine</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Red Oak</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Sugar Maple</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>White Oak</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Striped Maple</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Paper Birch</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chestnut Oak</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>White Ash</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Green Ash</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Red Spruce</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4. A breakdown of the number of plots on which each species was found and the mean basal area (BA) for each species when present.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Uninfested</th>
<th>Infested</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HWA and Decline Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Infestation</td>
<td>0 (0)</td>
<td>36 (2.85)</td>
<td>1 - 100</td>
<td>*</td>
</tr>
<tr>
<td>Decline Rating</td>
<td>2.26 (0.07)</td>
<td>3.55 (0.11)</td>
<td>0.75 - 7.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Years Infested</td>
<td>0 (0)</td>
<td>5 (0.31)</td>
<td>0 - 11</td>
<td>*</td>
</tr>
<tr>
<td>% New Growth</td>
<td>81 (1.80)</td>
<td>62 (2.56)</td>
<td>2 - 100</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Transparancy</td>
<td>12 (0.51)</td>
<td>19 (0.81)</td>
<td>4 - 67</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Fine Twig Dieback</td>
<td>4 (0.29)</td>
<td>8 (0.63)</td>
<td>0 - 35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% Living Crown</td>
<td>61 (1.21)</td>
<td>57 (1.23)</td>
<td>16 - 93</td>
<td>0.0133</td>
</tr>
<tr>
<td><strong>Foliar Chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (mg kg⁻¹)</td>
<td>5038 (170.03)</td>
<td>6063 (244.95)</td>
<td>2416 - 16369</td>
<td>0.0011</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>6837 (82.97)</td>
<td>7420 (108.18)</td>
<td>4405 - 10614</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>1265 (25.16)</td>
<td>1275 (37.28)</td>
<td>622 - 3583</td>
<td>0.833</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>828 (45.85)</td>
<td>1434 (74.88)</td>
<td>94 - 3699</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>1476 (38.38)</td>
<td>1786 (45.59)</td>
<td>923 - 3910</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N (Percent)</td>
<td>1.46 (0.01)</td>
<td>1.54 (0.02)</td>
<td>1.12 - 1.94</td>
<td>0.0002</td>
</tr>
<tr>
<td><strong>Stand Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total BA (m² ha⁻¹)</td>
<td>758 (14.78)</td>
<td>658 (10.72)</td>
<td>365 - 920</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>% EH</td>
<td>73 (1.20)</td>
<td>72 (1.01)</td>
<td>48 - 95</td>
<td>0.6907</td>
</tr>
<tr>
<td>% Dead EH</td>
<td>7 (0.56)</td>
<td>12 (1.28)</td>
<td>0 - 55</td>
<td>0.0014</td>
</tr>
<tr>
<td>Mean EH BA (m² ha⁻¹)</td>
<td>21 (0.54)</td>
<td>23 (0.73)</td>
<td>14 - 53</td>
<td>0.0386</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>329 (21.56)</td>
<td>237 (12.90)</td>
<td>102 - 1710</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mean EH Height (m)</td>
<td>26 (0.43)</td>
<td>28 (0.45)</td>
<td>16 - 41</td>
<td>0.0009</td>
</tr>
<tr>
<td><strong>Climate Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Winter T (°C)</td>
<td>24 (0.29)</td>
<td>27 (0.24)</td>
<td>23 - 32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current Drought Impact</td>
<td>2 (0.11)</td>
<td>2 (0.06)</td>
<td>2 - 4</td>
<td>0.4865</td>
</tr>
</tbody>
</table>

Table 5. A summary of measured variables on infested and uninfested plots. Significant t-tests (p < 0.01) are in bold and are based on DF = 44.

The average decline rating for infested trees was 3.5. This was significantly higher than the 2.3 rating for uninfested trees (Table 5). Percent infestation ranged from 0 to 100, with an average of 36% on infested plots. Infested plots ranged from 0 to 55% dead hemlock, with an average of 12% dead standing basal area; this was significantly more than the 7% average for uninfested plots (Table 5).
Foliar Chemistry

Percent infestation was positively correlated with foliar Ca, K, Mn, N and P concentrations (Table 6). Partial correlations on these significant variables indicated that the strongest relationships were between infestation and N, Ca and P (Table 6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>P</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Infestation</td>
<td>0.18</td>
<td>0.24</td>
<td>0.09</td>
<td>0.35</td>
<td>0.35</td>
<td>0.26</td>
</tr>
<tr>
<td>Decline Rating</td>
<td>0.34</td>
<td>0.22</td>
<td>0.03</td>
<td>0.34</td>
<td>0.46</td>
<td>0.11</td>
</tr>
<tr>
<td>% Transparency</td>
<td>-0.04</td>
<td>0.38</td>
<td>-0.25</td>
<td>0.11</td>
<td>0.43</td>
<td>0.20</td>
</tr>
<tr>
<td>% Fine Twig Dieback</td>
<td>0.02</td>
<td>0.31</td>
<td>-0.08</td>
<td>-0.03</td>
<td>0.34</td>
<td>0.17</td>
</tr>
<tr>
<td>% New Growth</td>
<td>-0.70</td>
<td>0.21</td>
<td>-0.36</td>
<td>-0.56</td>
<td>-0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>% Living Crown</td>
<td>0.03</td>
<td>-0.19</td>
<td>0.11</td>
<td>-0.07</td>
<td>-0.37</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

**Table 6. A. Correlations based on the 225-sample regional data set highlight significant relationships (p < 0.01) between foliar chemistry, infestation and decline parameters. B. Partial correlations for only the significant pairwise variables are also included in order to quantify the relationships with infestation and decline independent of multi-collinearity effects.**

The number of years that a tree was infested was the strongest pairwise correlate with hemlock decline symptoms (r = 0.6356, p < 0.0001). Because of this relationship, many of the variables associated with HWA infestation are also significantly correlated.
with hemlock decline. More severe decline symptoms were associated with higher concentrations of Ca, K, Mn and P (Table 6). Partial correlations indicated that Ca and P had the strongest association with hemlock decline (Table 6). Examining individual health measurements, transparency, fine twig dieback and new growth were most closely correlated with P, and percent new growth with Ca. All correlations with decline parameters were positive (Table 6).

**Predictive Models**

One-degree factorial linear regressions were used to evaluate how much of the overall variability in infestation and health witnessed in the regional study could be accounted for by the key foliar chemistry variables identified in the multiple tiers of this study (N, K, Ca and P). With an $R^2$ of 0.31 and RMSE of 22.72 (Figure 8), infestation could be predicted with 10% tolerance with 94% accuracy. The hemlock decline regression built only on Ca, K and P reported an $R^2$ of 0.43 and RMSE of 0.85 (Figure 8) with a 1-class tolerance accuracy of 93%.
Figure 8. Linear regression models to predict HWA infestation levels and hemlock decline exemplify how much of the variability can be explained using only N, K, P and Ca.

\[
\%\text{Infestation} = -117.8 + (Ca \times 0.003) + (K \times 0.003) - ((Ca - 5573) \times (K - 7128) \times 0.001) + (P \times 0.01) + (N \times 51.3) - ((P - 1630) \times (N - 1.5) \times 0.09) 
\]

\[
\text{Health} = -1.6 + (Ca \times 0.001) + (K \times 0.001) - ((Ca - 5579) \times (K - 7126) \times 4.8) + (P \times 0.001) 
\]
A stepwise linear regression model based only on landscape characteristics was able to predict hemlock decline with an $R^2 = 0.37$, RMSE = 0.90 and a 1-class tolerance accuracy of 90%.

A stepwise linear regression based only on landscape characteristics (as identified as significant factors in previous research (Royle and Lathrop 1999) (Orwig et al. 2002) (Mayer et al. 2002) selected a model based on the percent infestation, precipitation, percent hemlock basal area, aspect and landscape position. This 5-term model reported an $R^2$ of 0.37 and RMSE of 0.90 (Figure 9), with a 1-class tolerance accuracy of 90%.
Figure 10. The best-fit predictive model included both a mix of landscape and chemistry variables. This 8 term model was able to predict decline with an $R^2 = 0.68$, RMSE = 0.64 and 98% 1-class tolerance accuracy.

Allowing the mixed stepwise regression to select from all possible chemical and landscape variables produced the best model, with an $R^2 = 0.68$, RMSE = 0.64 and 98% 1-class tolerance accuracy. The final set of variables instrumental in predicted decline across the Northeast included: the duration of HWA infestation, precipitation, percent hemlock basal area, aspect, landscape position, Ca, P, N and cellulose concentrations.
Discussion

A complex suite of biological, chemical and environmental variables governs the suitability of a host tree. Several studies (Bonneau et al. 1997; Mayer et al. 2002; Royle and Lathrop 1999; Orwig et al. 2002) have shown that site and climatic factors, such as winter temperatures, landscape position and aspect, may influence hemlock decline on HWA infested trees. However, based on these results, we suggest that the inclusion of chemical factors is essential in understanding regional infestation and decline patterns.

Although multiple elements were significant in each of the individual parts of this study, only Ca, N, K and P were significant in all three tiers and will be discussed further.

Species Comparison

Foliar chemistry was measured for resistant hemlock species and compared to that of eastern hemlock. Although adelgid does infest Asian hemlock species, occasionally attaining high densities, the trees are typically not significantly injured (McClure M.S. et al. 1996). The same has been reported for western species where HWA is primarily found only on trees stressed by other factors (McClure M.S. et al. 1996). In contrast, eastern hemlock has shown little or no resistance, with many trees succumbing within four years of initial infestation.

If some species of hemlock are resistant to HWA, and if foliar chemistry plays a part in this resistance, then one would expect to see inherent differences in foliar chemistry that are similar when comparing all resistant to non-resistant species. Both N and K were significantly higher in eastern hemlock than in resistant Asian species. Since the Asian species have evolved with, and adapted to HWA, this implies that there may be some benefit to having lower N and K. Although K data was not available for western
North American species, the same relationship was evident when comparing foliar N between TSCA and resistant western species (Figure 6).

The opposite relationship may exist for P and Ca. While Ca concentrations were higher in the Asian than North American species, this was only significant for comparisons to TSSI. This difference was striking, with Ca levels more than 3 times higher in the Asian species (Figure 5). At the Arnold Arboretum, TSCA foliar P was also only significantly different from TSSI, with lower concentrations in the Asian than North American species. However, comparisons of the East/West Dataset suggest that resistant species growing within their native range have significantly higher P concentrations (Figure 6).

### Colonization Study

We hypothesized that HWA would be unable to establish a viable population on the resistant Asian and Western species. However, foliar chemistry (not species, where p = 0.39, or resistance class, where p = 0.97) was significantly associated with the number of live sistens present at the end of the colonization study.

Following the hypothesis that there may be some benefit to maintaining low concentrations of N and K (as demonstrated by resistant species), K was the strongest correlate with the number of live sistens present and also highly correlated with the overall number of ovisacs (a good indicator of reproductive success over the course of the study). Initial N concentrations were also strongly linked to the number of old ovisacs present (Table 3). Higher concentrations of each of these elements were associated with greater colonization success, regardless of host species.
This indicates that there could be a palatability effect with N and K. There is a large body of evidence to support this hypothesis. Nitrogen has been shown to be positively associated with aphid success for a variety of host species (Carrow and Betts 1973; McClure 1980; Koritsas and Garsed 1985; Douglas 1993). Specific to HWA, N fertilization resulted in increased relative growth rate, survivorship and fecundity (McClure 1991; McClure 1992). McClure (1992) also found that nitrogen fertilization increased eastern hemlock susceptibility to HWA and reduced the effectiveness of implanted and injected pesticides.

This suggests that inherently low N concentration may limit HWA success, which in turn may impart some measure of resistance for host trees. Nitrogen is particularly important to insects because there exists a significant difference between the nitrogen concentration of plants (around 2% dry weight) and that of insects (approaching 7%) (Dale 1988). Concerning relatively immobile insects, such as HWA, the nutritive quality of forage becomes even more important. Under low nitrogen conditions, concentrations may not be sufficient to maintain viable HWA populations. McClure (1991) attributed the larger number of HWA eggs taking the winged sexuparae form to higher population densities and deteriorating host nutrient qualities. This represents a major mortality factor for adelgid due to the lack of suitable alternate spruce host (McClure 1991).

Results from the literature are more mixed for K, indicating that the relationship between the element and resistance may be host/insect specific. Some studies concerning various insect herbivores find no appreciable effect of K on overall insect success (Kairo and Murphy 1999; Elden and Kenworthy 1995) Others have found that increased K
imparts resistance to aphid species (Rohilla et al. 1993). Still others have found that there appears to be an increase in K concentration from primary producer to consumers, highlighting the importance of K in herbivore nutrition (Risley 1990). These data suggest that similar to N, K may be a nutritional requirement for herbivores and a limiting factor to HWA population growth.

There were also significant relationships between Ca, P and colonization success. Initial concentrations of Ca and post-infestation P were the strongest correlates with the number of live sistens present at the end of the study (Table 3). Trees with the highest concentrations of these elements were either unsuccessfully infested, or maintained very low HWA population levels, regardless of species.

Similar to these results, Harada et al. (1996) found that resistant tobacco species had Ca contents 10-100 times higher than aphid susceptible species. Further study isolated exudates of CaCl₂ as having anti-aphid activity, and named CaCl₂ as the driving factor in tobacco resistance to green peach aphids (Myzus persicae). They concluded that CaCl₂ is toxic to aphids rather than simply a feeding deterrent. Chhillar and Verma (1985) also identified Ca as contributing towards aphid resistance in barley plants.

Previous research has also shown that applications of P fertilizers have abated the effects of Alphis craccivora (Roch) on cowpea plants (Annan et al. 1997). Phosphorus was also identified as one of the factors contributing to aphid resistance in Brassicae crops (Rohilla et al. 1993; Harada et al. 1996; Chhillar and Verma 1985). Specifically, Marzo et al. (1997) found a close correlation between different P application rates and phytic acid levels in various pea varieties, and a linear correlation between phytic acid
content and *Bruchus pisorum* infestation. They concluded that greater phytate content could reduce the risk of *Bruchus* infestation in pea seeds.

Previous research, combined with data from this study, suggests that some defensive strategy involving Ca and P may be involved, and that locations with high supplies of these nutrients may impart some degree of resistance to eastern hemlock.

We acknowledge that the Arboretum samples used in the species comparison and colonization study were taken from landscape specimens growing under different conditions than what is typically experienced by wild trees. Further, because trees are tended on an individual basis, this design does not qualify as a tightly controlled "garden" experiment. However, the culmination of evidence, including comparisons of native eastern and western species, indicates that while foliar chemistry absolute concentrations may be off for landscape specimens, the general relationships between chemistry and HWA success are still meaningful.

**Regional Study**

The regional data support a forage quality, palatability based hypothesis with N as the strongest partial correlate with percent HWA infestation on eastern hemlock trees across their native range (Table 6, Figure 11). Higher N concentrations lead to higher infestation levels, with host palatability (via N content) driving HWA population dynamics. This in turn leads to more severe hemlock decline symptoms. This is witnessed in the regional study where foliar transparency and fine twig dieback are associated with higher N concentrations (Table 6). Based on these findings, it is possible that eastern hemlock stands with relatively higher N nutrient status may be predisposed to catastrophic infestations, ultimately leading to their demise. This may be most common
in mixed stands with higher quality litter inputs from deciduous species, or in areas approaching N saturation from atmospheric inputs (Aber et al. 1998; McNulty et al. 1991).

Figure 11. ANOVA shows a significant difference between infested and uninfested groups for nitrogen (p = 0.0002), calcium (p = 0.0011) and phosphorus (p < 0.0001). Cumulative frequency plots show the highest infestation levels (black) are typically associated with the highest nitrogen and mid to lower calcium and phosphorus concentrations.

Potassium as a causal factor is also backed up by the regional study. Potassium was significantly correlated with both HWA infestation levels and hemlock decline symptoms across the Northeast (Table 6). While existing literature on K and insect
infestations (Kairo and Murphy 1999; Rohilla et al. 1993) is not as clear as that for N, it is plausible that a strong link exists between foliar K and HWA success and warrants further investigation.

Higher concentrations of Ca in infested trees could be attributed to a defensive response similar to what was reported by Harada et al. (1996) in tobacco plants and may prove to be significant over a longer time frame. A defensive response hypothesis could explain why Ca was positively correlated with infestation across the regional study (Table 6). Even though the infested group as a whole had higher Ca concentrations, the trees with the highest infestation levels had some of the lowest Ca concentrations (Figure 11).

Ca was also positively correlated with hemlock decline across the regional study. However, it is more likely that this association with decline is a result of the close correlation between the percent new growth (a factor in decline calculations) and foliar Ca. As foliage ages, Ca oxalate accumulates in the foliage as a means to neutralize the oxalic acid byproducts of photosynthesis. Therefore, the close association between hemlock decline and Ca concentrations is most likely a result of differences between new and old growth foliage and their proportions in healthy vs. declining trees.

Because significant changes in P were not detectable over the course of this study, a palatability relationship is implied with higher P acting as a deterrent to herbivory by HWA. Similar to Ca, higher concentrations of P were positively associated with higher infestation percentages and increasing decline symptoms (Table 6), yet negatively associated with colonization success (Table 3). While this positive correlation with infestation across the regional study appears contrary to a feeding deterrent type
relationship, we also found that while infested trees do have higher P as a group, the highest infestation levels are actually associated with mid to low P concentrations (Figure 11).

The strong positive correlation between P and decline symptoms is also contrary to a feeding deterrent hypothesis. However, it is possible that P concentrations have not reached a level necessary to impede HWA feeding in eastern hemlock. This seems plausible when comparing the average P concentration of 2248 ppm for resistant species (Table 1) to 1786 ppm for infested eastern hemlock in the infested group (Table 5). While these results are not as clear as for Ca, the idea of P acting as a feeding deterrent is compatible with previous work, which has shown the negative effects of P on various aphid pests (McLaughlin 2001; Rohilla et al. 1993; Harada et al. 1996; Chhillar and Verma 1985).

In order to estimate how much of the variability in infestation levels and hemlock decline can be attributed to foliar chemistry, multiple linear regressions were applied using only elements that were significant in all three tiers of this study. While all of the key elements identified here were significant in predicting infestation and decline, the low $R^2$ values (Figure 8) indicate that additional variability remains to be explained. This indicates that while foliar chemistry is significant in the evaluation HWA infestation intensities and decline symptoms, the inclusion of both chemistry and site variables (as reported by previous studies (Bonneau et al. 1997) may help to more fully explain the highly variable infestation and decline patterns witnessed in the field.
**Predictive Models**

Previous research has primarily implicated site factors related to water availability in patterns of hemlock decline (Royle and Lathrop 1999; Orwig et al. 2002; Mayer et al. 2002). Similar to this work, we found that several site factors were significantly associated with hemlock decline and could be used to predict decline across the Northeast. This 5-term model showed more severe decline symptoms with higher infestation levels, drier than normal growing season, higher hemlock stocking, southern and western exposures, and ridge top/side-slope positions. This is all in agreement with previous work indicating that water availability may be an additional stressor, speeding decline. It could also be that such drier conditions lead to a concentration of high palatability nutrients in sap and foliage. This could also drive HWA population levels.

The limitation is that, with an $R^2 = 0.37$, even including HWA infestation (the number 1 correlate with health), a landscape characteristics based model is only able to account for a little over one third of the variability in decline (Figure 9). This is less than the almost half of variability explained by the 5 term chemistry model (Figure 8).

Obviously these approaches are not mutually exclusive and when we allow the model to select from all potential variables, the same landscape variables are retained with the addition of Ca, P, N and cellulose (Figure 9). This final model is able to account for over two thirds of the variability in hemlock decline across the Northeast. This indicates that the addition of chemistry data should greatly improve the predictive ability of any HWA susceptibility models.
Conclusions

The multiple components of this research indicate that a suite of macronutrients is strongly correlated with adelgid infestation. These results support the host nutritional quality hypothesis for foliar N and K, implicating both as causative agents, positively associated with HWA population levels and increasing hemlock decline symptoms. We further conclude that foliar Ca and P could have negative impacts on HWA population levels. While the mechanisms of this relationship are not clear, P and Ca may be involved in a defensive response to HWA infestation, with higher concentrations potentially imparting some degree of host resistance, with tolerance for prolonged, low level infestations.

While these findings do not isolate the exact mechanisms of the relationships between foliar nutrient elements and HWA assemblages, the connection is clear. A closer examination of foliar chemistry on a landscape scale may help interpret patterns of hemlock decline and mortality witnessed in the field, which is critical in ultimately controlling this outbreak.
CHAPTER II.

ASSESSING HEMLOCK DECLINE USING VISIBLE AND NIR SPECTROSCOPY: SIGNATURE ANALYSIS, INDICES COMPARISON AND ALGORITHM DEVELOPMENT

Abstract

Data from an ASD FieldSpec Pro spectrometer was used to determine if narrow-band foliar reflectance can be used to assess detailed hemlock decline. Key wavelengths from signature analyses and established stress indices were used in a stepwise linear regression for comparison to a full visible and near infrared spectrum MPLS regression on a 400 sample calibration set of fresh foliage collected from across the Northeast. A 6-term linear regression (including: Carter Miller Stress Index ($\frac{R_{694}}{R_{760}}$), Derivative Chlorophyll Index ($\frac{FD_{705}}{FD_{723}}$), Normalized Difference Vegetation Index ($\frac{R_{800} - R_{680}}{R_{800} + R_{680}}$), R950, R1922 and FD1388) was able to predict the 0-10 decline scale on an independent validation set with an $R^2$ of 0.71 and root mean square error of 0.591. Treated as a class variable, this predicted the 11-class decline rating with a one-class tolerance accuracy of 97%. Based on stress induced reductions in chlorophyll content and function, healthy trees and those in early decline (pre-visual symptoms) were differentiated with 72% accuracy, indicating that hyperspectral sensors could be used to detect the very early stages of hemlock decline.
Introduction

One of the most pressing forest health issues currently facing North American forests is the widespread decline of eastern hemlock (*Tsuga canadensis* Carriere) due to the hemlock wooly adelgid (HWA, *Adelges tsugae* Annand). Since the 1980's when HWA was introduced to the northeastern U.S., it has spread rapidly leading to decline and mortality from North Carolina to Massachusetts (Souto et al. 1995).

Most assessments of decline following HWA infestation involve time-consuming field based methods. Although these methods are valuable in monitoring gross changes over time, they are not able to identify trees in the very early stages of decline (Sampson et al. 2000). By the time a hemlock stand exhibits symptoms of HWA infestation detectable using field based surveys, the stand may already be adversely impacted. The ability to identify trees in the very early stages of decline at the landscape scale will assist in the development of integrated pest management strategies aimed at controlling HWA infestation.

There is mounting evidence that narrow-band hyperspectral instruments have the capability to not only assess defoliation, but also identify the early signs of stress, in some cases before visual symptoms are apparent (Cibula and Carter 1992; Carter 1993; Carter and Miller 1994; Mohammed et al. 1995; Zarco-Tejada et al. 2000a; Zarco-Tejada et al. 2000c). This can be explained by the tendency of stressed leaves to experience reduction in photosynthetic activity and chlorophyll content. Even subtle changes can alter reflectance across the visible and NIR spectrum (Carter and Knapp 2001; Rock et al. 1988; Vogelmann and Rock 1988; Gitelson and Merzlyak 1996; Vogelmann et al. 1993).
Chlorophyll $a$ and $b$ content are particularly good detectors of stress because of their direct role in photosynthesis. Narrow wavebands near 700 nm where changes in chlorophyll absorption are easily detectable have been recommended for early detection of forest damage (Hoque et al. 1990; Hoque et al. 1992) and were used to detect decreased vigor, before visual symptoms were apparent, in pine seedling canopies (Cibula and Carter 1992). Because changes in chlorophyll function typically precede changes in chlorophyll content, chlorophyll fluorescence has also been shown to be a useful tool in identifying pre-visual strain (Zarco-Tejada et al. 2000a; Zarco-Tejada et al. 2000c).

Such diagnostic features for estimating plant decline are located in relatively narrow wavebands, interspersed with insensitive features (Treitz and Howarth 1999). Ratios or pairs of wavelengths (indices) tend to highlight significant features while correcting for geometrical and background effects (Baret and Guyot 1991). This is accomplished by targeting stress sensitive bands, while including an insensitive “control” band which functions as a baseline to exclude variability due to other factors (Treitz and Howarth 1999). Such simple transformations have been closely correlated with plant characteristics without the sensitivity to external variables such as sun angle or instrument variability (Pinty et al. 1993).

Hyperspectral spectrometers measure many contiguous narrow spectral bands, which allow for the application of analytical techniques based on absorption features in the observed materials. Given that airborne or spaceborne hyperspectral remote sensing imagery is difficult to acquire and process, it is necessary to first determine whether
hyperspectral reflectance data could be useful in detecting stands infested by HWA at the leaf level.

This study examines the relationship between benchtop hyperspectral reflectance data from fresh hemlock foliage and hemlock decline across the Northeast. Our objectives were to: (1) determine which wavelengths and/or stress indices were most strongly correlated with hemlock decline, (2) use this information to develop a simple linear equation to predict decline using a minimal number of variables, and 3) compare the predictive abilities of the equation developed in #2 to a modified partial least squares regression (MPLS) based on the entire visible and NIR spectrum.

**Methods**

In 2001, 45 HWA monitoring plots (20m x 20m) were established across the northeastern U.S. in Connecticut, Massachusetts, New Hampshire, New Jersey, New York and Pennsylvania (Figure 4). These plots were resampled in 2002 with the addition of 12 more plots in the Catskills, New York. Plots were selected to cover a range of infestation levels and histories, hemlock decline symptoms and site nutrient status.

Five canopy dominant hemlocks were selected as study trees from each plot and sampled in mid to late October of each year. Foliage was collected from the mid and upper canopies of each tree using a 12-gauge shotgun. For each tree, the foliage was combined and immediately sealed, refrigerated and transported back to the lab for analysis. Hemlock decline was assessed using methods specifically designed to quantify the various sequential symptoms of hemlock decline that follow adelgid infestation. This included the percent of terminal branchlets with new growth (R. Evans pers. comm.), percent transparency (quantified using a concave spherical densiometer (Pontius et al. (1999) Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.)
2002)), percent fine twig dieback and live crown ratio (USDA Forest Service Crown Rating Guide). The categories of hemlock decline described in Table 1 reflect the typical characteristics of each measurement at various stages of hemlock decline. Decline category assignments for each measurement were averaged to determine one overall decline rating that best described the trees’ overall status (where 0 = perfect health, 10 = dead).

**Spectral Data Collection**

Fresh foliage from each sample tree was stacked to opacity on a 0.3m diameter, spinning platform. Hyperspectral reflectance data was collected using an ASD FieldSpec Pro FR field spectroradiometer (Analytical Spectral Devices, Boulder, CO). The light source and spectrometer were both angled at 45° toward the stacked sample (Figure 12). Averaging spectra from 12 scans minimized instrument and sampling error, with the sample rotated 30° between scans. Following the same protocol, every fifth sample included measurements from a reference panel, in order to take a ratio with the preceding sample data to calculate relative reflectance.
Full Spectrum Calibration

A randomly selected 400-sample subset of the combined 2001/2002 data was used to create the hemlock decline predictive equations. The remaining 100 samples were retained for independent validation. Raw reflectance, first, second and third derivative spectra were considered with a variety of segment intervals and smooth pre-treatments. Each of these variations on the PLS regression was run using Unscrambler version 7.6 SR-1 (CAMO Technologies).

Final equation statistics were derived from full cross validation, where the regression was re-run to the exclusion of each sample iteratively. In this way, each sample was treated as an unknown and predicted once during equation development.
Equations were evaluated based on the standard error of calibrations (SEC), calibration correlation and standard error of prediction (SEP) generated from full cross validation during equation development. The SEC is an estimate of the best accuracy obtainable using the given input and constituent data (Mark and Workman 1991). However, the SEP can be viewed as a more accurate representation of the calibration’s prediction accuracy on unknown samples (Westerhaus 1989). The equation with the lowest SEP was used to predict hemlock decline on a 100-sample independent validation set to determine its functionality with independent data.

**Signature Analysis**

In addition to the partial least squares regression, we examined existing spectral indices known to assess physiological symptoms of plant stress (Table 7). These indices were calculated from our sample data and made available for selection in a mixed, stepwise linear regression to predict hemlock decline.
<table>
<thead>
<tr>
<th>Index</th>
<th>Formula</th>
<th>Primary Absorbance Feature</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carter and Miller Stress CMS</td>
<td>$CMS = \frac{R_{694,nm}}{R_{760,nm}}$</td>
<td>Chlorophyll Content</td>
<td>Carter and Miller 1994</td>
</tr>
<tr>
<td>Curvature Index CI</td>
<td>$CI = \frac{R_{683,nm} + 2}{R_{675,nm} \times R_{691,nm}}$</td>
<td>Chlorophyll Fluorescence</td>
<td>Zarco-Tejada et al. 2002</td>
</tr>
<tr>
<td>Derivative Chlorophyll Index DCI</td>
<td>$DCI = \frac{FD_{705,nm}}{FD_{723,nm}}$</td>
<td>Chlorophyll a &amp; b Content; Chlorophyll Fluorescence</td>
<td>Zarco-Tejada et al. 2002</td>
</tr>
<tr>
<td>Chlorophyll Fluorescence CF</td>
<td>$CF = \frac{FD_{690,nm}}{FD_{735,nm}}$</td>
<td>Chlorophyll Fluorescence; Photosynthetic Activity</td>
<td>Mohammed et al. 1995</td>
</tr>
<tr>
<td>Normalized Difference Vegetation Index NDVI</td>
<td>$NDVI = \frac{R_{800,nm} - R_{680,nm}}{R_{800,nm} + R_{680,nm}}$</td>
<td>Chlorophyll Content and Energy Absorption</td>
<td>Deblonde &amp; Cihlar 1993; Gamon et al. 1997; Myneni et al. 1995; Rouse et al. 1974</td>
</tr>
<tr>
<td>Photo-Chemical Reflectance Index PRI</td>
<td>$PRI = \frac{R_{531,nm} - R_{570,nm}}{R_{531,nm} + R_{570,nm}}$</td>
<td>Xanthophyll Cycle Activity</td>
<td>Gamon et al. 1990; Gamon et al. 1997; Rahman et al. 2001</td>
</tr>
<tr>
<td>Red Edge Inflection Point REIP</td>
<td>$REIP = \lambda_{FD_{\max}}$</td>
<td>Chlorophyll a Content; Green Vegetation Density</td>
<td>Gitelson et al. 1996; Rock et al. 1988; Vogelmann et al. 1993</td>
</tr>
<tr>
<td>Ratio Vegetation Index RVI</td>
<td>$RVI = \frac{R_{800,nm}}{R_{680,nm}}$</td>
<td>Chlorophyll Content</td>
<td>Pearson and Miller 1972; Royal and Lathrop 2001</td>
</tr>
<tr>
<td>Water Band Index WBI</td>
<td>$WBI = \frac{R_{970,nm}}{R_{900,nm}}$</td>
<td>Canopy Water Content</td>
<td>Carter 1993; Penuelas et al. 1997; Tucker 1980</td>
</tr>
</tbody>
</table>

Table 7. A list of existing indices included in our analyses that are known to have strong relationships with stress specific physiological responses (i.e. reductions in photosynthesis or chlorophyll content).

In order to identify potentially new spectral regions that may contribute to decline predictions, analyses of the ASD data also included examinations of raw, first second and third derivative spectra by decline class. First, the response decline variable was translated into a categorical decline class (by rounding to the nearest integer). Average reflectance was examined to identify spectral regions where decline classes were significantly distinct. The average spectral reflectance of each decline class was also divided by the reflectance of healthy, non-stressed samples so that wavelengths of maximum sensitivity could be identified regardless of reflectance intensity (Fractional Difference).
Because the use of derivatives enhances small peaks that may be masked by broadband features or background noise, we also included the analysis of first, second and third derivative spectra calculated with a gap interval of 4nm. In this way, even weaker signals could be isolated (Dixit and Ram 1985).

**Statistical Analyses**

Pairwise multiple correlations were used to examine relationships with hemlock decline for all wavelengths and indices identified in the signature analyses. Hsu’s MCB multiple means comparisons was used to determine at which decline class indices were first able to differentiate significant \( p < 0.01 \) differences in spectra (Hsu 1996).

A mixed, stepwise regression was then used to select the best linear regression model for the 400-sample calibration set. To avoid over fitting, conservative significance cutoff limits were established for each of the regression steps (probability to enter = 0.250, probability to leave = 0.01). Mallow’s Cp and PRESS statistics were used to compare the predictive abilities of various models (Kleinbaum et al. 1998). The final selected model was then used to predict decline on the independent validation set (100 samples). All correlations, means comparisons and linear regression techniques were conducted using JMPin software version 4.0.3 (Sall et al. 2001).

**Results**

**MPLS Regression**

Of the various spectral math pre-treatments, an 8-term MPLS regression based on the raw reflectance data generated the lowest SEP at 1.06 and an \( R^2 = 0.50 \). Treating decline as a class variable by rounding to the nearest integer resulted in a one-class
tolerance accuracy of 89% on the independent validation set (Table 8, Figure 13). The wavelengths with the highest coefficients in the MPLS regression equation were located at 552nm, 702nm, 800nm, 1396nm and 1922nm (Figure 14). These were also significantly correlated with decline in pairwise comparisons (Table 9).

<table>
<thead>
<tr>
<th>Model</th>
<th>R²</th>
<th>RMSE</th>
<th>Terms</th>
<th>Accuracy</th>
<th>Within 1</th>
<th>Within 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPLS Full Spectrum</td>
<td>0.50</td>
<td>0.82</td>
<td>8</td>
<td>36</td>
<td>89</td>
<td>97</td>
</tr>
<tr>
<td>Linear Combined Index</td>
<td>0.67</td>
<td>0.82</td>
<td>5</td>
<td>45</td>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>CMS Only Linear Model</td>
<td>0.36</td>
<td>1.07</td>
<td>1</td>
<td>40</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td>DCI Only Linear Model</td>
<td>0.13</td>
<td>1.24</td>
<td>1</td>
<td>26</td>
<td>58</td>
<td>82</td>
</tr>
<tr>
<td>FD 1388nm Only Linear Model</td>
<td>0.23</td>
<td>1.30</td>
<td>1</td>
<td>31</td>
<td>58</td>
<td>81</td>
</tr>
<tr>
<td>R 950nm Only Linear Model</td>
<td>0.03</td>
<td>1.31</td>
<td>1</td>
<td>28</td>
<td>61</td>
<td>82</td>
</tr>
<tr>
<td>R 1922nm only Linear Model</td>
<td>0.07</td>
<td>1.35</td>
<td>1</td>
<td>28</td>
<td>48</td>
<td>70</td>
</tr>
</tbody>
</table>

Table 8. Results from independent validation using various full spectrum and index regression models. The best results were obtained for the 5-term linear combined index equation indicating that a combination of key wavelengths and established indices can accurately predict a detailed health rating system. By rounding decline to the nearest integer, accuracy results are also presented within 1 and 2 classes for the ten class rating system.
Figure 13. An independent validation set was used to test the predictive abilities of the MPLS regression model. This resulted in an $R^2$ of 0.50 and RMSE of 0.82. Treated as a class variable, the MPLS regression predicted decline with a one-class tolerance accuracy of 89%. 

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Figure 14. Regression coefficients from the MPLS regression of raw reflectance spectra. Higher (absolute value) coefficients signify wavelengths with more information related to decline.

**Signature Analysis**

An analysis of the fractional difference between healthy and declining samples (Figure 15) standardizes reflectance to a healthy sample baseline and therefore helps to highlight the wavelengths in which reflectance varies most significantly with stress regardless of reflectance magnitude. From these significant wavelengths, a mixed stepwise linear regression selected reflectance at 950nm and 1922nm for inclusion in decline predictions (Table 9) as instrumental in predicting decline. Wavelengths at 680nm and 800nm (used to calculate the Normalized Difference Vegetation Index (NDVI), and 694nm and 760nm (used to calculate the Carter and Miller Stress Index (CMS) were also identified for use in the predictive equation (Table 9).
Figure 15. The fractional difference highlights those areas where declining samples differ from typical reflectance for healthy samples (class 1 and 2 as the baseline at 1.00). Reflectance at 680nm, 694nm, 760nm, 800nm, 950nm and 1922nm were instrumental in predicting decline.

Because there is an inherent baseline shift in absolute reflectance between healthy samples and sparse declining samples, we also examined derivative transformations of the raw reflectance data, which transcend differences in raw reflectance (Figure 16). First derivative transforms at 1388nm, as well as the Derivative Chlorophyll Index (DCI), were significantly correlated with decline and retained in the final predictive model (Table 9). Second and third derivative spectra did not contribute significantly to decline predictions.
Figure 16. First derivative spectra of the red edge inflection point (left) and the 1388nm region (right) highlight wavelengths key in predicting hemlock decline.

**Indices**

Each of the vegetation indices included in this study was significantly correlated with hemlock decline (p < 0.01), with generally stronger correlations than for the individual wavelengths from the signature analyses (Table 9). The strongest relationships were found with CMS and Chlorophyll Florescence (CF). The best detectors of early stress were CMS, the Ratio Vegetation Index (RVI), CF and NDVI (Table 9). Each of these indices was able to pick up a significant difference from healthy samples by decline class 4.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation with Decline</th>
<th>First Differentiates Declining from Healthy</th>
<th>Absorbance Feature</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carter and Miller Stress</td>
<td>0.5503</td>
<td>4</td>
<td>Chlorophyll Content</td>
<td>Carter and Miller 1994</td>
</tr>
<tr>
<td>Chlorophyll Fluorescence</td>
<td>0.5149</td>
<td>4</td>
<td>Chlorophyll Fluorescence; Photosynthetic Activity</td>
<td>Mohammed et al. 1995</td>
</tr>
<tr>
<td>Water Band Index</td>
<td>0.4708</td>
<td>6</td>
<td>Canopy Water Content</td>
<td>Carter 1993; Penuelas et al. 1997; Tucker 1980</td>
</tr>
<tr>
<td>Ratio Vegetation Index</td>
<td>-0.4484</td>
<td>4</td>
<td>Chlorophyll Content</td>
<td>Pearson and Miller 1972</td>
</tr>
<tr>
<td>Red Edge Inflection Point</td>
<td>-0.3627</td>
<td>5</td>
<td>Chlorophyll a Content; Green Vegetation Density</td>
<td>Gitelson et al 1996; Rock et al. 1988; Vogelmann et al. 1993</td>
</tr>
<tr>
<td>Derivative Chlorophyll Index</td>
<td>0.3521</td>
<td>5</td>
<td>Chlorophyll a &amp; b Content; Chlorophyll Florescence</td>
<td>Zarco-Tejada et al. 2002</td>
</tr>
<tr>
<td>R800</td>
<td>-0.3906</td>
<td>3</td>
<td>R-NH₂</td>
<td>Osborne and Fearn 1986</td>
</tr>
<tr>
<td>R1922</td>
<td>0.2669</td>
<td>5</td>
<td>CONH and Total Nitrogen Content</td>
<td>Bolster et al. 1996; Shenk et al 1992; Osborne and Fearn 1986 Deblonde &amp; Cihlar 1993; Gamon et al. 1997; Myneni et al. 1996; Bolster et al. 1996; Shenk et al 1992</td>
</tr>
<tr>
<td>Normalized Difference Vegetation Index</td>
<td>0.2429</td>
<td>4</td>
<td>Chlorophyll Content and Energy Absorption</td>
<td>Carter and Miller 1994</td>
</tr>
<tr>
<td>FD 1388</td>
<td>-0.2122</td>
<td>3</td>
<td>CH₃</td>
<td>Carter 1993; Carter and Knapp 2001</td>
</tr>
<tr>
<td>R1396</td>
<td>-0.1919</td>
<td>3</td>
<td>CH₂</td>
<td>Shenk et al 1992; Osborne and Fearn 1986</td>
</tr>
<tr>
<td>Curvature Index</td>
<td>0.1902</td>
<td>4</td>
<td>Chlorophyll Fluorescence</td>
<td>Zarco-Tejada et al. 2002</td>
</tr>
<tr>
<td>R552</td>
<td>0.1845</td>
<td>7</td>
<td>Chlorophyll a</td>
<td>Carter 1993</td>
</tr>
<tr>
<td>Photo-Chemical Reflectance Index</td>
<td>0.1634</td>
<td>4</td>
<td>Xanthophyll Cycle Activity</td>
<td>Gamon et al. 1996; Gamon et al. 1997; Rahman et al. 2001</td>
</tr>
<tr>
<td>R702</td>
<td>0.1441</td>
<td>7</td>
<td>Chlorophyll a</td>
<td>Carter 1993; Carter and Knapp 2001</td>
</tr>
<tr>
<td>R950</td>
<td>-0.1347</td>
<td>3</td>
<td>Water</td>
<td>Williams and Norris 2001</td>
</tr>
</tbody>
</table>

All correlations are significant at the p < 0.01 level.

Table 9. Key variables from the MPLS and linear regressions are defined, including pairwise correlations with decline, the decline class first significantly different from healthy samples and known absorbance features. Variables are listed by correlation strength.
**Linear Regression**

A five-term equation for predicting hemlock decline was created using a stepwise linear regression technique (Equation 1). Treating decline as a class variable, this equation was able to predict decline class with 45% accuracy and within one class with 96% accuracy on the independent validation set (Figure 17). The combined equation performed significantly better than any of the individual wavelengths (Table 8) and with a maximum variance inflation factor of 1.8, showed little instability due to correlation among terms.

\[ \text{Decline} = 0.29 + (9.11 \times CMS) + (1.78 \times DCI) - (3.42 \times R950) + (26.75 \times R1922) - (95.39 \times AD388) \]

**Equation 1.** The ASD FieldSpec Pro linear regression equation for predicting hemlock decline, where CMS = R694/R760, DCI = FD705/FD723). Validation statistics were: \( R^2 = 0.67, \text{RMS} = 0.82. \)
Figure 17. A linear regression based on CMS, DCI, NDVI, reflectance at 950 and 1922 nm and the first derivative at 1388nm was tested on an independent validation set. Decline rating was predicted with an $R^2 = 0.67$ and RMSE = 0.82. Converting this data to a class variable showed 96% one-class tolerance accuracy.

Discussion

These results indicate that hyperspectral reflectance data can be used to predict hemlock decline. The simple linear regression based on six terms worked as well as the full spectrum MPLS regression (Table 8). The key variables in both of these equations (Equation 1, Figure 14, Figure 18) have been related to health or physiological function in various plant species.
Figure 18. A close look at the visible and NIR portions of the spectra highlight those wavelengths found to be significant in predicting hemlock decline. Wavelengths in gray were key to the MPLS regression, while wavelengths in black were used in the linear equation.
The most heavily weighted variables are located at chlorophyll absorption bands (Table 9), including R552nm, R702nm, R680nm (as a component of NDVI), 705nm and 723nm (as components of the DCI Index) (Carter 1993; Carter and Knapp 2001; Kolling et al. 1996; Zarco-Tejada et al. 2002).

Chlorophyll content is a good indicator of stress because of its direct role in photosynthesis. Increasing reflectance near the 700 nm range represents the often reported blue shift in stressed plants, resulting from stress induced reductions in chlorophyll content (Rock et al. 1988; Cibula and Carter 1992).

Because the absorptivity of chlorophyll is relatively low in this region, even small decreases in chlorophyll content results in significantly decreased absorption and increased reflectance in stressed plants (Carter 1993; Carter and Knapp 2001). Changes in reflectance in response to stress have been directly linked to foliar chlorophyll content in numerous studies (Rock et al. 1988; Gitelson and Merzlyak 1996; Vogelmann et al. 1993).

The indices retained for inclusion in our index regression make use of several key wavelengths representative of chlorophyll florescence, including 680nm (NDVI Index), 694nm and 760nm (Carter Stress Index), and 705nm and 723nm (DCI Index) (Carter and Miller 1994; Zarco-Tejada et al. 2002; Carter and Knapp 2001). When photosynthetic tissues are excited with sunlight, excess light is emitted in order to dissipate light energy that exceeds photosynthetic requirements. The resulting florescence is inversely related to actual photosynthetic rates (D'Ambrosio et al. 1992; Larcher 1994; Schreiber and Bilger 1994), making it a good measure of relative photosynthetic activity. CF may help to identify decline before visual symptoms are apparent because changes in chlorophyll
function often precede changes in chlorophyll content. Direct measurement of CF is a well-established method for examining physiological function. Relating CF emissions to leaf reflectance, Zarco-Tejada et al. (Zarco-Tejada et al. 2002) found that CF emissions impart a detectable signature on leaf reflectance data in the red edge region (680 nm - 800 nm).

Significant in both the index (as a component of NDVI) and the MPLS regressions is reflectance at 800 nm. Absorbance at this location is associated with the aliphatic amine \( \text{R-NH}_2 \). This functional group is a primary component of the chemical structures involved in plant energy dynamics (i.e. ATP, NAD and NADP), as well as proteins essential to plant metabolism and growth (Hopkins 1999). While it is conceivable that stress may cause changes in plant proteins and energetics, because of its prevalence in many chemical structures, it is difficult to directly link these results to a specific chemical or physiological stress response.

Similarly, the methylene group \( \text{CH}_2 \), associated with absorbance at 1396 nm from the MPLS regression and FD 1388 from the index regression (Shenk et al. 1992), is associated with numerous chemical structures in plants. However, methylene groups are a common component of chlorophyll \( a \) (Hopkins 1999), concentrations of which have been strongly linked to plant stress levels (Rock et al. 1988). Methylene is also a component of various plant growth regulators (e.g. auxins and gibberellins) that are synthesized in young, developing foliage (Hopkins 1999). Based on the reductions in new growth associated with increasing decline ratings, this could also explain the significance of reflectance at this location.
A strong water absorption band at 950nm was also included in the index regression (Williams and Norris 2001). Relative susceptibility of hemlock to HWA has been linked to various site and landscape factors related to water availability (Borneau et al. 1997; Onken 1995; Royle and Lathrop 1999), with drier conditions stressing already weakened trees. Although we did not measure leaf water content, it is plausible that trees experiencing the most significant decline may be suffering from both infestation and water stress. There is also evidence that HWA injects toxic saliva at feeding sites (McClure M.S. et al. 1996). It is postulated that the toxic effects of this saliva may include a constricting effect on xylem, which could lead to leaf dehydration following infestation (Shields et al. 1995). While Carter (Carter 1993) concluded that infrared reflectance responds consistently only when stress has developed sufficiently to cause severe leaf dehydration, we did see significant differences between our decline classes at 950nm where water is the primary absorber.

Reflectance at 1922nm is attributed to absorbance of C-O-N-H peptide bonds. Bolster et al. (Bolster et al. 1996) found that this location was significant in predicting total leaf nitrogen and cellulose content. There is evidence that nitrogen concentration in foliage is linked to adelgid population levels in a positive forage quality based relationship (McClure 1991). In turn, HWA population dynamics are the number one determinant of hemlock health within the infestation front (Pontius et al. unpublished data.). It then follows that reflectance at an N sensitive band could be linked to decline symptoms.

Cellulose is a common component of cell walls and could reflect the relative integrity of foliar tissues, which degrade as decline progresses. While both of these
connections to hemlock decline are loose, it is not surprising that reflectance at 1922nm was significant in both the MPLS and index regressions.

**Identification of Pre-Visual Decline Symptoms**

In order to determine when visual symptoms become noticeable in terms of the rating system used in this study, individual measurements were averaged for each decline category (Figure 19). Of the measurements that comprise this rating system, the first response to infestation is a reduction in the production of new growth. Such subtle changes are not readily apparent in the field except at the early stages of spring flush. We begin to see a sharp drop in the percent new growth between decline classes 3 and 4 (Figure 19).

The second symptom of HWA infestation is needle loss (measured as canopy transparency) and fine twig dieback. Most forest health ratings are based on similar defoliation measurements, categorizing the percent defoliation into 5 or 10 percent classes by ocular estimation (USDA Forest Service Crown Rating Guide). Both transparency and fine twig dieback increase steadily across the entire decline rating range. However, up until decline class 4, both measurements stay below what is typically first categorized as defoliation (fine twig dieback below 5 percent and transparency below 15 percent) (Figure 19).
The most obvious, and typically the last response to infestation, is dieback of branches and limbs. This begins with the loss of heavily shaded understory branches, and is quantified as a reduction in the percent live crown. While some dieback due to shading is common even among healthy trees, the steep drop after decline class 6 is where severe adelgid induced decline becomes obvious.

These results indicate that visual symptoms would most likely not be noticed until decline class 4 when new growth drops precipitously and fine twig dieback first reaches
the 5 percent class typically recorded as the first indication of decline. Therefore, any results that are successfully able to differentiate between healthy samples and samples ranked as a decline category 4 are most likely picking up changes in tree health before visual symptoms are apparent.

Because several of the key variables we identified were successful at differentiating between healthy samples (class 1 or 2) and samples with pre-visual decline symptoms (class 4) (Table 9), it is possible that these indices could be used to detect strain before symptoms are apparent in the field.

It is our belief that this model could be applied to remotely sensed hyperspectral data in order to classify hemlock health and early decline on a landscape scale. Because independent validation was even stronger for the simple linear regression than for the full MPLS regression, narrow-band instruments fitted with filters to target several key wavelengths (Table 9) may work just as well as full hyperspectral instruments with significantly lower attainment costs and processing time. The accuracy and detail attained with narrow band models is superior to time consuming field based methods, and should be able to detect decline sooner than aerial photography or the coarse resolution multi-spectral instruments that are typically used to assess forest health on a landscape scale.

**Conclusions**

These results indicate that a simple linear regression model based 5 terms is able to accurately predict a detailed hemlock decline rating system (97% one-class tolerance accuracy). Primarily based on stress-induced changes in chlorophyll content and function, this model was able to predict decline class on an independent validation set.
with a one-class tolerance accuracy of 97%. The accuracy of differentiating between healthy samples (class 1 and 2) and those in early decline (class 3 and 4, pre-visual symptoms) was 72%, indicating that hyperspectral sensors could be used to detect trees in the very early stages of decline.

These findings suggest that techniques could be developed that use hyperspectral remote sensing instruments to detect early stages of HWA infestation at the landscape scale. Such techniques would provide a much-needed tool for the early detection of stressors such as HWA infestation, and will allow forest land management agencies to focus biological control efforts on incipient infestations before trees are severely impacted. In conjunction with existing methods for mapping foliar chemistry (Martin and Aber 1997; Hallett et al. 1997; Smith et al. 2002), development of this capability could greatly enhance our ability to detect patterns of hemlock susceptibility, adelgid infestation limits and hemlock decline across large spatial scales.
CHAPTER III

USING AVIRIS TO ASSESS HEMLOCK ABUNDANCE AND EARLY DECLINE IN THE CATSKILLS, NEW YORK

Abstract

In order to aid land managers in monitoring and controlling the ongoing hemlock woolly adelgid (HWA) outbreak, more accurate landscape scale tools are required to identify infestation and early decline. To this end, NASA’s AVIRIS instrument was flown over the infestation front in the Catskills region of New York during the summer of 2001. Spectral unmixing produced a hemlock abundance map with a 20 percent tolerance accuracy of 64%. This correctly identified hemlock dominated pixels (>50% basal area) with 88% accuracy. Key wavelengths and health indices were examined to determine if a subset of narrowband wavelengths could accurately predict an 11-class decline rating system. Reflectance at a chlorophyll sensitive wavelength (683nm), coupled with a water band index (R970/900), was able to predict decline with 85% accuracy. The extreme accuracy at the low (0-4) end of the range indicated that these wavelengths might be used to assess early decline, before visual symptoms are apparent.
Introduction

Over the past century, one of the greatest threats to the health of North American forests has been the introduction of exotic pathogens and insect pests. Such invasions often result in drastic and long-term changes in forest ecosystems, aesthetic conditions and important natural resources (Liebhold et al. 1995; Castello et al. 1995). During the 20th century, widespread mortality and decline has occurred among species such as American chestnut (*Castanea dentata* Marsh), American elm (*Ulmus Americana* L.), oak (*Quercus* spp.) and American beech (*Fagus grandifolia* Ehrh.). Currently, eastern hemlock (*Tsuga canadensis* (L. Carr.) forests are threatened by another major forest pest, the hemlock woolly adelgid (*Adelges tsugae* Annand) (HWA).

Since the 1980's when HWA first surfaced in the Northeast, it has spread rapidly, leading to decline and mortality in over eleven states, from North Carolina to Massachusetts (Souto et al. 1995) (Figure 2). Although its trajectory is somewhat unpredictable, current estimates are that HWA is spreading 10 – 15 miles a year into uninfested areas (Orwig and Foster 1998; Souto et al. 1995). Once settled on young hemlock twigs, HWA causes needle loss, bud mortality and finally branch and tree mortality, sometimes within four years (McClure 1991; Shields et al. 1995). Most infested eastern hemlock trees have shown no resistance to HWA and little chance for recovery (McClure 1995a).

The potentially severe consequences and large scale of the HWA infestation requires that land managers be familiar with the actual location of the hemlock resource and its health and infestation status. Most assessments of forest decline involve time-consuming field based methods. Although these methods are valuable in monitoring
gross changes over time, they are not able to identify trees in the very early stages of decline (Sampson et al. 2000) or assess large acreages. Remote sensing technologies are the most viable option to assist land managers in health assessment and monitoring at a regional scale.

To date, remote sensing of forest health has involved the classification of various degrees of defoliation and mortality using aerial photography or coarse spectral resolution visible/NIR space-based sensors, such as Landsat Thematic Mapper (TM). Lambert et al. (1995) used Landsat-TM imagery to separate three categories of damage in Norway spruce with 75% accuracy. Similar defoliation based studies have predicted four classes of hemlock defoliation with moderate accuracy (Royle and Lathrop 2002; Royle et al. 1995; Royle and Lathrop 1997).

The use of this type of imagery is limited in several ways. For example, in areas of mixed coniferous vegetation, errors can be introduced due to the spectral similarity between damaged trees of one species to healthy spectra of other species (Rosengren and Ekstrand 1988). In addition it can be equally difficult to distinguish a severely defoliated hemlock stand from an inherently sparse healthy stand (Royle and Lathrop 2002). Finally, when decline is measured solely as a function of defoliation, earlier signs of stress such as reductions in photosynthesis are not detected. When Landsat-TM data is used to assess forest health, moderate and light damage stands are often difficult to distinguish due to overlap in their reflectance ranges (Lambert et al. 1995); (Royle and Lathrop 2002).

Many studies have demonstrated that instruments with higher spectral resolution are needed to accurately detect changes in vegetation condition (Treitz and Howarth...
1999). Several diagnostic features for estimating plant decline are located in relatively narrow wavebands, interspersed with insensitive features (Treitz and Howarth 1999). Ratios or pairs of wavelengths (indices) tend to highlight significant features while correcting for geometrical and background effects (Baret and Guyot 1991) by targeting stress sensitive bands paired with an insensitive “control” band (Treitz and Howarth 1999). Such simple transformations have been closely correlated with plant characteristics, without the sensitivity to external variables such as sun angle or instrument variability (Pinty et al. 1993).

Common stress responses, such as the “blue shift” away from the normal inflection point of the red edge reflectance feature, are also dependent on high spectral resolution to pick up the subtle shifts (on the order of 5nm) that accompany pre-visual strain (Rock et al. 1988).

Using a 72-channel CASI sensor, Sampson et al. (2000) found strong relationships between reflectance and leaf chlorophyll content. Because chlorophyll content is known to decrease in stressed vegetation, it may be one of the most important indicators of early strain (Zarco-Tejada et al. 2000b). Chlorophyll $a$ and $b$ content are particularly good indicators of stress because of their direct role in photosynthesis. Narrow wavebands near 700nm where changes in chlorophyll absorption are easily detectable have been recommended for early detection of forest damage (Hoque et al. 1990; Hoque et al. 1992) and were able to detect decreased vigor, before visual symptoms were apparent, in pine seedling canopies (Cibula and Carter 1992).

Preliminary unpublished work by the authors using an ASD FieldSpec Pro FR field spectroradiometer (Analytical Spectral Devices) highlight several indices and
wavelengths that are able to track hemlock stress, including pre-visual symptoms (CHAPTER II). This study was designed to determine if hyperspectral remote sensing imagery could be used to predict early hemlock decline symptoms in the Catskills State Park, New York.

**Methods**

**Remote Sensing Data**

On July 20, 2001, hyperspectral data from NASA’s Airborne Visible/Infrared Imaging Spectrometer (AVIRIS) was obtained for the 700,000 acre Catskills State Park region. Flown on an ER-2 aircraft, AVIRIS measures upwelling radiance in 224 contiguous channels between 400nm and 2400nm with a 10nm spectral resolution (Vane et al. 1988). In October 2001, hemlock decline data was collected at 13 HWA monitoring plots (20m x 20m) and percent hemlock basal area data recorded at 81 prism plots across the Catskills. Geographic location data was collected for all plots using a WAAS enabled Merridian Gold global positioning system (Rees 2001).

Between the sensor and the surface, there is an extremely dynamic atmosphere that can dramatically alter the spectral radiation reflected from the canopy (Schowengerdt 1997). In order to account for absorption and scattering by gasses and particulates as light passes through the atmosphere, atmospheric calibration algorithms using radiative transfer codes, rescale raw radiance data to surface reflectance by correcting for atmospheric influence (Van der Meer and de Jong 2001). Atmospheric Correction Now software (ACORN 4.0 Analytical Imaging and Geophysics LLC, ImSpec 2002), developed specifically for hyperspectral sensors such as AVIRIS, removed atmospheric
effects in the data using averaged time and geographic data inputs from the five individual AVIRIS runs.

With a field of view of 30 degrees, AVIRIS samples with view zenith angles of up to 15 degrees to each side of nadir. This causes a nearly linear decrease in reflectance as the view angle moves from forelit to backlit surfaces (Kennedy et al. 1997). To remove the resulting view-angle brightness gradient while maintaining the radiometric integrity of the image, the AVIRIS imagery was corrected using a CSIRO destreak algorithm (Datt et al. 2003), modified by the authors to employ global statistics averaged from all 5 runs. This algorithm uses an empirical correction applied by normalizing mean and variance for each view angle.

After atmospheric and brightness corrections, the imagery was geometrically registered to a USGS 1m resolution digital orthoquads using a polynomial degree 2 warping method (ENVI 4.0 software, Research Systems, Inc). AVIRIS reflectance spectra were then extracted for pixels covering each of the 13 decline and 81 abundance plots (Figure 20).
Figure 20. The steps involved in processing the raw AVIRIS imagery and then creating the hemlock abundance and decline coverages over the Catskills.

**Hemlock Abundance**

Seventy field plots were randomly selected from primarily evergreen dominated areas within the AVIRIS imagery to determine the percent hemlock basal area using a basal area factor 10 prism. Mixture Tuned Matched Filtering (MTMF), a special case of spectral mixture analysis (Williams and Hunt 2002); (Nielsen 2001); (Tompkins et al. 1997) was conducted using a 28-band Minimum Noise Fraction transformed image matched to 7 “pure hemlock” calibration pixels with greater than 90% hemlock cover. Probability and infeasibility data were averaged from the 4 pixels adjacent to prism point locations for input to a 2-term linear regression. The resulting equation was then applied to the AVIRIS image on a pixel-by-pixel basis.

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Hemlock Decline

The five canopy dominant hemlocks on the 13 established monitoring plots were assessed using methods specifically designed to quantify the various sequential symptoms of decline that follow adelgid infestation. This included the percent of terminal branchlets with new growth (R. Evans pers. comm.), percent transparency (quantified using a concave spherical densiometer (Pontius et al. 2002)), percent fine twig dieback and live crown ratio (USDA Forest Service Crown Rating Guide). The categories of hemlock health described in Table 1 reflect the typical characteristics for each health measurement at various categories of hemlock decline. Health category assignments for each measurement on a tree were averaged to determine one overall decline rating that best described tree health (where 0 = perfect health, 10 = dead). Health scores were averaged for the five canopy dominant trees on each plot to determine a continuous plot level health rating for comparison to hyperspectral imagery.

Previously established stress detecting indices (Table 7) were entered into a stepwise linear regression, along with a suite of individual wavelengths significantly correlated with hemlock decline in a previous ASD FieldSpec Pro benchtop study (CHAPTER II). Due to the small sample size, a maximum of 2 terms and conservative significance cutoff limits were established for each of the regression steps to avoid over fitting (probability to enter = 0.250, probability to leave = 0.01). Mallow’s Cp and PRESS statistics were used to compare the predictive abilities of various models (Kozak and Kozak 2003). Because of the small sample size, full double-cross validation (jackknifed residuals) was used in lieu of independent validation to assess predictive abilities (Kozak and Kozak 2003). After establishing the best-fit hemlock decline model,
the resulting equation was applied to all pixels with greater than 40% hemlock basal area on a pixel-by-pixel basis.

**Results and Discussion**

**Hemlock Abundance**

Percent hemlock basal area ranged from 0 to 100 percent on the 70 abundance plots, with an average of 49 percent (Table 10). Of these plots, the majority were dominated by evergreen species, with only 13 percent classified as hardwood or non-forested types (Table 10). Because of the domination of evergreen stands in this data set, this becomes a strong test of how well hemlock can be differentiated from other spectrally similar species.

<table>
<thead>
<tr>
<th>Type</th>
<th>N</th>
<th>Avg. %HE</th>
<th>Min %HE</th>
<th>Max %HE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>Hemlock Mix</td>
<td>26</td>
<td>54</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>Hemlock</td>
<td>21</td>
<td>86</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Larch</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Red Spruce Mix</td>
<td>4</td>
<td>48</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>White / Red Pine</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 10. Of the 70 basal area prism points used to validate the MTMF for hemlock abundance, the vast majority were dominated by evergreen species. These points cover a range from 0 to 100 percent hemlock with an average of 49% hemlock basal area.
Output from the MTMF included a) the probability that the pure hemlock signature was a component of reflectance and b) the infeasibility of the pure hemlock signature as a component of reflectance for each pixel. These 2 variables were input into Equation 2 to calibrate percent hemlock basal area across the 70 prism plots. This translation of MTMF data to abundance reported an $R^2$ of 0.65 and a RMSE of 12 (Figure 21). For comparison to straight classifications of dominant cover type, the MTMF based regression was able to correctly differentiate hemlock/non-hemlock (cutoff at 40% hemlock basal area) stands 83% of the time.
Abundance = 76.10 + (Prob * 41.18) − (Infeas * 5.53)

Equation 2. The linear regression equation to predict percent hemlock basal area where Prob = the probability output and Infeas = the infeasibility output from a MTMF process. Validation statistics were: \( R^2 = 0.65 \) and \( \text{RMSE} = 12 \).

Figure 21. Hemlock abundance was predicted with an \( R^2 \) of 0.65 and RMSE of 12. Most error was manifest as under predicting stands with significant understory hemlock (bottom right gray) and pine dominated stands predicted to have hemlock (upper left gray). Circles represent hemlock and mixed hemlock stands, open squares are dominated by other evergreen species. Hemlock dominated stands were differentiated from non-hemlock stands with 83% accuracy.
Much of the error introduced in this model resulted from pine-dominated stands predicted to have a higher percentage of hemlock than is actually present (Figure 21). The spectral unmixing methods appear to be able to identify all dominant hemlock stands accurately, however, there is also the risk of classifying dense, white pine stands as containing some hemlock when they do not. The most erroneous prism plots used in this validation include red pine plantation stands surrounded by mixed hemlock forest. It is our belief that this problem may not be so much a limitation of the AVIRIS instrument as a georegistration issue in areas of severe topography. While this error is significant in our abundance predictions, we believe that this equation adequately identifies hemlock dominated stands for the application of decline predictions.

In the upper end of the range, hemlock abundance predictions were often low (Figure 21). This may be due to the inclusion of understory hemlock in the prism plot percent basal area measurements that are not visible to the AVIRIS sensor. In mixed, mid-successional stands, hemlock may not be mature and present in the canopy in numbers sufficient to impact canopy spectral signatures. Such trees are likely shaded out by the intermediate successional, mature hardwood or pine canopy dominants. Mickelson et al. (1998) found similar problems with mixed hemlock stands using multi-temporal Landsat TM data.

For problems such as the HWA induced hemlock decline, it is useful to map actual hemlock abundance so that areas of relative susceptibility and ecological impact can be identified. Applying Equation 2 to the full AVIRIS image (Figure 22) highlights the heavy concentration of hemlock in riparian areas. This suggests that widespread
hemlock decline and mortality could be a significant factor in deteriorating surface water quality in the Catskills.

Figure 22. A map of percent hemlock basal area highlights the high concentration of hemlock in lowland and riparian areas. Circles represent independent prism plot validation points.

**Hemlock Decline**

While multiple factors were significantly correlated with decline (Table 11), a mixed, stepwise linear regression suggests that only R683 and the Water Band Index (WBI) (Carter 1993); (Penuelas et al. 1997); (Tucker 1980) were necessary to predict decline ($R^2 = 0.88$, RMSE = 0.23 and a PRESS = 1.67) (Equation 3; Figure 23). Treated as a class variable by rounding to the nearest integer, this predicted decline class with 85% accuracy and to within one class with 100% accuracy. These wavelengths were also
significantly correlated with decline (WBI $r = 0.47$, R683 $r = 0.27$) in a previous leaf-level study (CHAPTER II).

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Correlation with Decline</th>
<th>Absorbance Feature</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R683</td>
<td>0.8542</td>
<td>Chlorophyl$_a$</td>
<td>Carter 1993</td>
</tr>
<tr>
<td>R1653</td>
<td>0.6508</td>
<td>Benzene Rings, C-H stretch</td>
<td>Williams and Norris 2001</td>
</tr>
<tr>
<td>R1304</td>
<td>0.5643</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>R952</td>
<td>0.4611</td>
<td>Water</td>
<td>Williams and Norris 2001</td>
</tr>
<tr>
<td>R760</td>
<td>0.4347</td>
<td>Water</td>
<td>Osborne and Feam 1986</td>
</tr>
<tr>
<td>CF = $\frac{FD693nm}{FD731nm}$</td>
<td>0.3992</td>
<td>Chlorophyll Florescence; Photosynthetic Activity</td>
<td>Mohammed et al. 1995</td>
</tr>
<tr>
<td>WBI = $\frac{R971nm}{R904nm}$</td>
<td>0.3787</td>
<td>Canopy Water Content</td>
<td>Carter 1993; Penuelas et al. 1997; Tucker 1980</td>
</tr>
</tbody>
</table>

Table 11. AVIRIS variables significantly (p < 0.20) correlated with decline and known absorbance features. Variables are listed by correlation strength. Only R683nm and the WBI were retained for inclusion in the stepwise linear regression to predict decline.
Figure 23. The two-term linear regression equation based on R683nm and the WBI predicted hemlock decline with an $R^2$ of 0.88 and RMSE of 0.23.

\[ \text{Decline} = -16.82 + (R683 \times 0.02) + (WBI \times 15.40) \]

Equation 3. The linear regression equation to predict hemlock decline where R683 = the reflectance at 683nm and WBI = the Water Band Index as defined in Table 11. Validation statistics were: $R^2 = 0.88$, RMSE = 0.23 and PRESS = 1.67.

Given that Equation 3 works well on an empirical basis, it is useful to understand whether the wavelengths and indices used in the regression equations are theoretically related to tree health or physiological function. The strongest correlation with decline, and one of the key terms in Equation 3, occurred at R683nm. This wavelength is

Chlorophyll content is a good detector of stress because of its direct role in photosynthesis. Increasing reflectance near the 700 nm range represents the often-reported blue shift in stressed plants. This shift in the red edge inflection point results from stress induced reductions in chlorophyll content (Rock et al. 1988; Cibula and Carter 1992). Because the absorptivity of chlorophyll is relatively low in this region, even small decreases in chlorophyll content result in significantly decreased absorption and increased reflectance in stressed plants (Carter 1993; Carter and Knapp 2001). Stress induced changes in reflectance have been directly linked to foliar chlorophyll content in numerous studies (Rock et al. 1988; Gitelson and Merzlyak 1996; Vogelmann et al. 1993).

The WBI was the second key term in predicting hemlock decline. This index is a ratio between the reflectance at 970nm, where absorbance by water is evident, and 900nm used as a reference, “control” band. Several studies have shown that the WBI closely tracks changes in leaf relative water content, leaf water potential and stomatal conductance (Bull 1991; Penuelas et al. 1993; Penuelas et al. 1994; Penuelas et al. 1996). In some species, WBI was able to track even mild water stress (Penuelas et al. 1996).

Relative susceptibility of hemlock to HWA has been linked to various site and landscape factors related to water availability (Bonneau et al. 1997); (Onken 1995); (Royle and Lathrop 1999). Drier conditions stress already weakened tress, making them more susceptible to HWA and decline. There is also evidence that HWA injects toxic saliva at feeding sites (McClure M.S. et al. 1996). It is postulated that the toxic effects of
this saliva may include a constricting effect on xylem, which could lead to leaf
dehydration following infestation (Shields et al. 1995). Although we did not directly
measure leaf water content, it is plausible that trees experiencing the most significant
decline may be suffering from some form of water stress.

The predictive decline equation (Equation 3) was created using spectra from
hemlock-dominated stands (40% to 100% hemlock). Therefore, we only predicted
hemlock decline for all pixels in the AVIRIS imagery with greater than 45 percent
hemlock as predicted by Equation 2. Predicted hemlock decline ranged from 0-7.7 across
the Catskills as compared to 1.8-4.1 found on the original 13 calibration plots (Figure
24).

Because HWA infestation was relatively new to the Catskills at the time the
imagery was collected, hemlock decline status does not complete the full range (0 to 10)
of decline rating. Ideally, calibration equations are developed with field data covering
the full range of possible conditions (Martin and Aber 1997). Using this same model on
leaf-level spectra from 100 independent validation trees across the Northeast from a
previous study (CHAPTER II) predicted tree level decline with a one-class tolerance
accuracy of 82%. Therefore, we believe that this equation should hold beyond the range
of decline available for calibration development.
Figure 24. Applied to all pixels with greater than 45 percent hemlock basal area, the decline prediction highlights more severe decline symptoms in the southeastern corner of the region where HWA has the longest history in hemlock stands. Other stressors are not excluded from this analysis.

More severe decline is evident in the southeastern region of the imagery, coinciding with the area first infested by HWA (Montgomery pers. com.). Average jackknifed residuals of 0.13 indicate that this equation should also work on independent data from the same range (Kozak and Kozak 2003). Additional data from 8 New York State Department of Environmental Conservation hemlock-monitoring plots was examined as a preliminary independent validation. While the DEC decline assessment was limited to a coarse evaluation of overall hemlock health (categorized as healthy,
HWA present but no visible damage, or discoloration), their assessments were predicted with a one-class tolerance accuracy of 88% (labeling healthy as decline class 2, HWA present but no visible damage as decline class 3 and discoloration as decline class 4). While these results are promising, a more rigorous validation covering the range of decline symptoms in the Catskills and matching our assessment methods is required to adequately represent the full range of conditions expected in natural hemlock stands.

HWA damage differs from many other forest stressors in that there is little noticeable change in color of damaged foliage (Royle and Lathrop 2002). Damage primarily appears as a gradual thinning of the foliage with tree death occurring in 2 to 4 years (McClure 1987). Defoliation does not reach levels classified as the very first stage of defoliation (>5% dieback or >15% transparency) by traditional field-based methods until after health class 3. Because Equation 3 works well below class 3, it appears that such imagery has the capability to identify decline pre-visual symptoms.

In order for this technology to be applicable on a large spatial and temporal scale, the relationships presented here must also be shown to work on other datasets. Because the AVIRIS data collected over the Catskills covered 5 different flight lines, we believe that the relationships presented here will prove robust enough for application to other narrow-band imagery. Further, because the initial selection of wavelengths for examination with the AVIRIS imagery was based on results from a previous ASD benchtop spectrometer, in which both studies showed similar relationships for key wavelengths and indices (CHAPTER II), we believe that these relationships will also hold across other narrow band instruments.
There is little evidence that these technologies can diagnose causal agents, as stress may be related to a variety of factors. Still, because the best hope of successful biological control for HWA must target newly infested areas where trees are still relatively healthy, the ability to identify stands early is essential to the development of sound forest management strategies.

Conclusions

These results show that a subset of narrow band remote sensing imagery can be used to predict hemlock decline at the landscape scale. The accuracy of the predictions below decline rating 3 indicates that these wavelengths may be capable of assessing previsual decline symptoms. Although this technique is not diagnostic, it is likely that most declining hemlock in this region is impacted by HWA. More research is required to assess the applicability of this equation to other sensors and across areas with a greater range of hemlock health.
FINAL CONCLUSIONS

The multiple components of this research indicate that a suite of macronutrients is strongly correlated with adelgid infestation. These results support the host nutritional quality hypothesis for foliar N and K, implicating both as causative agents, positively associated with HWA population levels and increasing hemlock decline symptoms. We further conclude that foliar Ca and P could have negative impacts on HWA population levels. While the mechanisms of this relationship are not clear, P and Ca may be involved in a defensive response to HWA infestation, with higher concentrations potentially imparting some degree of host resistance, with tolerance for prolonged, low level infestations.

Because changes in foliar chemistry are associated with decline, we were not surprised that spectral signatures also change with increasing decline symptoms. We found that a simple linear regression model based on 9 narrow band wavelengths is able to accurately predict a detailed hemlock decline rating system. Primarily based on stress-induced changes in chlorophyll content and function, this model was able to predict decline class on an independent validation set with a one-class tolerance accuracy of 97%. The accuracy of differentiating between healthy samples (class 1 and 2) and those in early decline (class 3 and 4, pre-visual symptoms) was 72%, indicating that hyperspectral sensors could be used to detect trees in the very early stages of decline.

Because none of these key wavelengths are at areas of know atmospheric distortion, these techniques were adapted to hyperspectral remote sensing data in order to
identify the early stages of HWA infestation at the landscape scale. Using a chlorophyll absorption band (R683nm) and a canopy water sensitive index (WBI), we were able to predict decline with a class tolerance accuracy of 100%. The accuracy of the predictions below decline rating 3 indicates that these wavelengths may be capable of assessing previsual decline symptoms.

These techniques would provide a much-needed tool for the early detection of stressors such as HWA infestation, and will allow forest land management agencies to focus biological control efforts on incipient infestations before trees are severely impacted. In conjunction with existing methods for mapping foliar chemistry (Martin and Aber 1997; Hallett et al. 1997; Smith 2000), development of this capability could greatly enhance our ability to detect patterns of hemlock susceptibility, adelgid infestation limits and hemlock decline across large spatial scales.


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McClure, M.S. 1987. Biology and control of hemlock woolly adelgid. Connecticut Agricultural Experiment Station. CT.


