ABSTRACT

Title of Document:	Hyperspectral Reflectance as an Indicator of Foliar Nutrient Levels in Hybrid Poplar Clone OP-367 Grown on Biosolid Amended Soil
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Trees of the genus *Populus* are fast growing trees that require considerable amounts of water and nutrients to meet physiological growth demands. The determination of correlations between hybrid poplar leaf spectral reflectance in the 325-1100 nm range, laboratory foliar analysis of leaf macronutrient and micronutrient concentrations, and leaf water potential datasets were analyzed using Full Cross-Validation and Test Set Models via the partial least squares (PLS) method of regression analysis. Based on an evaluation of the slope of the Predicted vs. Measured regression line, the root mean squared error (RMSE), and r-squared, the majority of the models constructed did not adequately model foliar concentrations from spectral data. However, the models for H, N, P, K, Cu and Al had values (slope of the Predicted vs. Measured regression line greater than 0.50 and r-squared values greater than 0.50 in at least one type of model) that warrant future study.

HYPERSPECTRAL REFLECTANCE AS AN INDICATOR OF FOLIAR NUTRIENT LEVELS IN HYBRID POPLAR CLONE OP-367 GROWN ON BIOSOLID AMMENDED SOIL

By

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Dedication

To Tonya

For Zack and Josh

Acknowledgements

The completion of this endeavor would not have been possible without the help and support of many people. I would like to thank my advisor Dr. Gary Felton, for advice and guidance as I journeyed down this path. I would like to thank Dr. David Tilley for allowing me access to his equipment. My family, especially my wife Tonya, has been instrumental in seeing me through this arduous process. I would also like to thank the many graduate students, both past and present, in the former Biological Resource Engineering Department. Your support, ideas, comments, and suggestions have aided me in more ways than you will ever realize.

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Introduction

The generation of wastes and their subsequent release into the environment is an issue that continues to be at the forefront of public interest. Increasing public and academic knowledge about the fate and impact of pollutants on the environment is pushing the reduction of pollutant levels in discharges and the development of new technologies for pollutant elimination, reduction, or mitigation. Specifically, the use of hybrid poplar trees as a treatment agent represents one possible means of nutrient utilization that is a politically, socially, economically, and environmentally acceptable alternative treatment method. Measuring the amounts of nutrients and water available to the hybrid poplar trees, reflected in foliar concentrations, during the course of the treatment process is necessary in order to ensure that the trees do not become either nutrient or water deficient. This is necessary to ensure that the trees grow large enough to be both successful at nutrient removal and economically viable.

Analysis of the visible and near infrared spectrum presents an opportunity to develop a baseline for the correlation between spectral reflectance in the visible and near infrared spectra, and nutrient and water stress levels in the hybrid poplar, clone OP-367, trees. Previous studies have shown that there are correlations between the spectral characteristics of various crops and wetland plants to the available amounts of nutrients and water. This project will attempt to develop a similar correlation for hybrid poplar trees that may be used to replace or enhance currently-used nutrient and water-stress management techniques.

Background and Literature Review

Blue Plains

Location and General Information

The Blue Plains wastewater treatment facility, one of the largest advanced wastewater treatment facilities in the world, is located in Washington DC along the Potomac River. It provides wastewater treatment services for over 2.2 million people in the District of Columbia and the surrounding areas of Virginia and Maryland. The Blue Plains facility is a combined storm water and wastewater treatment facility that is permitted to handle up to 1400 million liters per day (370 million gallons per day) of sewage during non-precipitation events. During snowmelts or rainstorms, the facility is permitted to handle up to 4.073 billion liters per day (1.076 billion gallons per day). This ensures that all wastewater receives at least some level of treatment. The average daily treatment volume is 1219 million liters (322 million gallons). At the current rate of growth, the Blue Plains facility will reach its capacity in 2010 (Uman, 2002).

Effluent Discharge Limits

Since the Blue Plains facility discharges into the Potomac River, a major tributary of the Chesapeake Bay, there are a number of limitations placed upon allowable discharge level of wastes contained in the wastewater effluent. Total suspended solids are limited to 7.0 mg/L, dissolved oxygen concentration must be at least 5.0 mg/L, carbonaceous biochemical oxygen demand is limited to 5.0 mg/L, and the pH must be in the range of 6.0 to 8.5 (Uman, 2002). In addition to these water

quality parameters, there are two major nutrients of concern: phosphorus and nitrogen.

Phosphorus

Total phosphorus (TP) is limited to 0.18 mg/L (Uman, 2002). Naturally occurring background levels of TP are generally less than 0.03 mg/L, and keeping the concentration of TP in the receiving waters below 0.5 mg/L is intended to prevent long-term eutrophication (Dunne and Leopold, 1978). Phosphorus levels limit the extent of downstream algal blooms since phosphorous is a major limiting nutrient in freshwater ecosystems. Along with phosphorus, an additional nutrient of concern is nitrogen.

Nitrogen

From a human health perspective, the level of nitrogen in the form of nitrate in water is of particular concern. High levels of nitrate present in drinking water may result in the potentially fatal condition Methemoglobinemia, commonly known as Blue Baby Syndrome. This occurs when nitrate contaminated water is ingested and converted into nitrite by the digestive system. This in turn induces the hemoglobin (Fe^{2+}) in red blood cells to oxidize into methemoglobin (Fe^{3+}) (Knobeloch et al., 2000). Methemoglobin does not carry oxygen, thus preventing the oxygenation of the blood. The decrease in blood oxygen results in the blue coloration. This condition is particularly deadly in infants due to the lower levels found in infants of the enzyme methemoglobin reductase, which converts methemoglobin back into hemoglobin (Knobeloch et al., 2000). Additionally, chronic exposure to nitrate contaminated water has been linked to cancer, thyroid disease, and diabetes (Knobeloch et al.,

2000). The Safe Drinking Water Act (1974) sets the federal standard for the concentration of nitrate in drinking water to 10 mg/l.

From an environmental perspective, the impact of high levels of nitrogen in wastewater effluent is similar to that of phosphorus in that it results in an increase in algal production by downstream aquatic ecosystems. Controlling nitrogen levels is especially important in tributaries of the Chesapeake Bay because nitrogen is a limiting nutrient in saltwater environments. The level of acceptable nitrogen found in the discharge effluent varies by season. Reducing nitrogen concentrations in the effluent increases the overall health of downstream ecosystems by limiting the amount of nitrogen available to algae. Fewer and less intense algal blooms are a direct result of lower nitrogen concentrations in the effluent. Therefore, it is prudent to limit the amount of nitrogen in the water available for uptake by algae, especially during the warmer months of May through October when algal production is high. This is reflected in the lower discharge permit levels for ammonia nitrogen of 4.2 mg/L during this time period (U.S. EPA, 2007). During the colder months of November through April when colder temperatures inhibit algal blooms, allowable ammonia nitrogen discharge limits increase to 11.1 mg/L from November 1-February 14 and to 12.8 mg/L from February 15-April 30 (U.S. EPA, 2007). Hence, more nitrogen may be discharged into the receiving waters of the Potomac River without immediately impacting downstream environments during the colder months. The goal for the Blue Plains facility is a yearly discharge average of 7.5 mg/L for total nitrogen (U.S. EPA, 2007). However, a large amount of the nitrogen and phosphorus

produced by the wastewater treatment processes at the Blue Plains facility is not discharged as effluent but ends up in a relatively solid form known as biosolids.

Biosolids

General Information

The generation of biosolids and their subsequent disposal represents a growing problem facing wastewater treatment facilities nation wide. Biosolids are a semi-solid product generated in the breakdown of the biological components of wastewater (Evanylo, 2001). On a dry weight basis, biosolids are typically composed of approximately 30% organic carbon, 2.5% total nitrogen, 1.8% total phosphorus, 1.1% total sulfur, 3.8% calcium, and less than 1% of the following: potassium, sodium, magnesium, iron, aluminum, copper, nickel, and zinc (vanLoon, 2000). On average, the Blue Plains facility produces over 1400 wet tons of biosolids per day. The biosolids produced by the Blue Plains facility are a dewatered, lime-stabilized Class B product. The addition of quicklime raises the pH of the biosolids to 11-12 while increasing the temperature within the biosolids, effectively killing or reducing microbe and pathogen populations to acceptable levels (Buswell, 2006). As of November 2008, the concentrations of heavy metals found in the biosolids produced at Blue Plains are below levels required by the U.S. EPA (1993) for exceptional quality limits under Title 40 of the Code of Federal Regulations Part 503 (Peot, 2008). Additionally, these heavy metal concentrations are lower than the 2025 concentration limits proposed by European Union (Peot, 2008). The composition of the biosolids produced by the Blue Plains facility and used at the ERCO tree plantation, based on a dry weight basis, is summarized in Table 1 (Buswell, 2006).

Table 1. Parameters for Blue Plains Biosolids

Parameter	Value
Moisture (%)	71.76
Solids (%)	28.24
N (%)	4.12
NH_4-N (%)	0.27
P_2O_5 (%)	2.99
K_2O (%)	0.41
Ca (%)	11.94
Mg (%)	0.31
S (%)	0.66
Mn (ppm)	173.55
Zn (ppm)	394.20
Cu (ppm)	207.42

Current Disposal Methods

As metropolitan populations, and their subsequent rates of waste generation, continue to increase, the disposal of increasingly larger amounts of biosolids, as well as their associated costs, becomes an issue facing wastewater treatment facilities. There are four main methods for the disposal for biosolids: composting, transport and disposal in landfills, incineration, or land application for agricultural use. Due to odor complaints, the composting facility formerly located in Montgomery County Maryland, was shut down in 1999. Thus, composting is not currently a disposal option in Maryland for the biosolids produced at Blue Plains. Transportation and landfill disposal are becoming increasingly economically and socially unacceptable disposal options due to increases in fuel costs and tipping fees. Rough estimates place the cost for disposing of biosolids in landfills at $60 per ton (Peot, C., personal communication, November 18, 2008). Regulations concerning air quality, especially carbon dioxide and fine particulates, and energy requirements are the two major concerns of incineration operations. In addition, the biosolids are not destroyed but reduced in volume. Thus, the ash produced at incineration operations must also be

disposed of, typically in a landfill. As a result, the majority of the biosolids produced at the Blue Plains facility are trucked to various locations throughout Virginia and Maryland and land applied as a nutrient source for various agricultural crops grown for use as animal feed. Rough estimates place the cost of land applying biosolids at $45 per wet ton (Peot, C., personal communication, November 18, 2008). At present, this is the lowest cost alternative for biosolids disposal. While biosolids do provide an important source of nutrients for crops, the amount of biosolids that may be applied as a crop nutrient supplement is strictly regulated.

Regulations

The use of biosolids for agricultural purposes dates back thousands of years. The ancient Chinese used night soil as a fertilizer for crops. Land application of biosolids is a regulated process under Part 503 of Title 40 of the Code of Federal Regulations (U.S. EPA, 1993). The goal of this rule is to limit the impact of biosolids on human health and the environment. This is achieved in part by limiting the concentrations of certain components in biosolids, specifically heavy metals and pathogen levels, and by using crop nutrient requirements, specifically for nitrogen but also for phosphorus, to determine the amount of biosolids that may be applied as a nutrient source for plants.

Agricultural Use and Benefits

The use of biosolids as a soil amendment does offer some advantages over typical fertilizers. Biosolids improve the overall health of the A-horizon in soil profile by serving as a carbon source and by offering additional capacity for moisture retention (Sopper, 1993). This is especially evident in high clay soils where the

addition of biosolids helps to increase soil pore spaces, making it easier for roots to penetrate and allowing for easier movement of water and air in the soil spaces (Evanylo, 2001). From an agricultural perspective, biosolids also help prevent potential nutrient shortages by providing a source of essential plant nutrients that are not commonly purchased due to the unknown or unpredictable nature of plant responses to these nutrients (Evanylo, 2001). These nutrients include sulfur, manganese, zinc, copper, iron, boron, and molybdenum. Despite the lower amounts of organic carbon found in lime stabilized biosolids when compared to anaerobically digested biosolids, this type of biosolid still helps increase soil health and plant fertility by neutralizing acidity, raising soil pH, and by providing a source of calcium (Evanylo, 2001; Stehouwer, 2003). However, the largest benefit provided to plants by biosolids is as a source of the key nutrient nitrogen (Stehouwer, 2003).

The total nitrogen content of typical biosolids ranges from 3%-5%, depending on the treatment methods used at the wastewater treatment plant (Stehouwer, 2003). There are two forms of nitrogen in biosolids: organic and mineralized. The majority of nitrogen in biosolids is in the organic form and must be mineralized by soil microbes into either nitrate or ammonium before it becomes available for plant uptake (Evanylo, 2001; Stehouwer, 2003). The smaller mineralized portion of nitrogen in biosolids is assumed to be immediately available for plant uptake. As previously mentioned, biosolids are also a source many other nutrients, making them an important potential source of nutrients for many different types of crops, from traditional agricultural plantings to newer silvicultural plantations.

Poplar

Classification

Poplars are members of the family *Salicaceae* (the Willow family) and the genus *Populus*. This genus contains 26 species. Representative species of the genus include the black cottonwood which is native to the Northwestern United States (*P. trichocarpa*), the eastern cottonwood which is a species native to the Eastern and Midwest of the United States (*P. deltoides*), the Lombardy poplar which was introduced from Europe and is now found throughout the United States (*P. nigra*), and the quaking aspen which is a native species found throughout the United States except the Southeast (*P. tremuloides*). Most members of this genus are found in riparian ecosystems and are native to northern hemisphere (Pearson, 2000).

General Physical Characteristics

Poplars are deciduous trees, and their leaves are simple, alternate, and triangular or heart shaped with many species having toothed leaf edges and veins that are pinnately patterned (Silberhorn, 1999). An example of poplar leaves is found in Figure 1. The lifespan of a typical poplar is from 50-100 years with some species living as long as 200 years. The typical range in height is from 24.4-57.9 meters (80-190 feet) at maturity. Poplars are one of the fastest growing tree species in North America. It is possible for a seedling to grow to a diameter at breast height (DBH) of over 38 centimeters (15 inches) in six years, with a typical poplar reaching a DBH of 91-152 centimeters (3-5 feet) at maturity (National Agroforestry Center, 2000; USDA, 2002). Poplar bark is gray in color smooth on younger trees, and becomes furrowed as the tree ages (USDA, 2002; Nesom, 2003). An important feature of

poplar bark is the presence of small cracks or pores in the bark called lenticels (Borman and Larson, 2002). These lenticels serve as a passive means of oxygen transport from areas of higher oxygen concentrations above ground to areas of lower oxygen concentrations found in the root zone (Nilsen and Orcutt, 1996). Poplars also have specialized tissue known as aerenchyma tissue that transports oxygen from the atmosphere to the roots, aiding root growth in low oxygen soil environments (Rieske et al., 2000). Transporting oxygen into the soil aids aerobic processes, such as the nitrification (Rieske et al., 2000). Though their roots may grow as deep as 50 feet in search of water, most poplars have an aggressive but shallow root system, with most roots in the upper 90 centimeters (3 feet) of the soil profile (St. John, 2001; Nesom 2003). This shallow root system makes poplars susceptible to being toppled by wind, heavy snow, or ice (Nesom, 2003). Figure 2 shows a poplar that was toppled by wind, exposing the shallow lateral root of the tree.

Figure 1. Poplar Leaves

Figure 2. Wind Toppled Poplar Showing Exposed Lateral Root

Methods of Reproduction

Poplars are dioecious trees and reproduce sexually by producing seeds or asexually through vegetative reproduction. Poplars are also known as cottonwood trees, and this nickname comes from the silky appearance of the fruit on the tree. The primary means of poplar reproduction is through the germination and establishment of windborne seeds. After about age seven, poplars produce flowers in the spring coinciding with rising water levels (Borman and Larson, 2002). After pollination, seeds are produced that are embedded in a matrix of fibers which assist in windborne or flotation long distance dispersion (Strauss et al., 1999). Seeds need specific environmental conditions, especially high levels of moisture and sunlight, for successful germination and survival, and the seeds have high levels of viability within the first five days following dispersal (Borman and Larson, 2002). Seed viability quickly decreases after this time, and seeds are viable for only up to two weeks under

natural conditions (Borman and Larson, 2002). The lack of long term viability means that poplars do not produce seed banks (Strauss et al., 1999). Competition from weeds and other plants after germination greatly reduces seedling survival rates until the trees reach age three and are able to shade out competition (Kuhn et al., 1998; Strauss et al., 1999).

Vegetative reproduction is the other method of reproduction used by poplars. This is a disturbance related method of reproduction. Fire, animal browsing, ice scouring, flood damage, wind, or snow damage may result in new individuals either through vigorous sprouting from stumps or through the rooting and growth of excised branches that land in favorable conditions on the ground. Most of the branches that break off are lateral branches, and the process of reproduction through the abscission of lateral branches is known as cladoptosis (Borman and Larson, 2002). Figure 3 shows an example of vegetative sprouting from a damaged poplar tree.

Figure 3. Poplar Vegetative Regrowth

Ecology

Poplars are an early succession species characterized by shade intolerance, drought intolerance, and a fast rate of growth (Borman and Larson, 2002; USDA, 2002; Nesom, 2003). These characteristic preclude the establishment of poplars in areas that have established herbaceous stands with a closed canopy (Strauss et al., 1999). Poplar species are found throughout the United States, but are more prevalent in riparian areas. Poplars readily establish on floodplains and other riparian areas due to the abundant levels of water and nutrients in these systems (Thomas et al., 2000; Nesom, 2003). Poplars also prefer the moist, well-drained sandy loam or silt loam

soils that tend to be found in riparian areas and do not do as well in soils that have high clay content (Heilman et al., 1995; Kuhn et al., 1998; Thomas et al., 2000). Adaptations to floodplain conditions are evident by the fact that poplars have an extensive root system which makes them resistant to flood damage and that their root systems are able to withstand periodic inundation while dormant (Kuhn et al., 1998; USDA, 2002). Additionally, poplars will grow on mountain slopes at altitudes less than 2100 meters (7000 feet) as long as there is adequate moisture present (Nesom, 2003).

Poplars are also quite important to many other species in the biomes in which they live. Large birds use the crowns of larger poplars as nesting sites (Nesom, 2003). The trunk cavities in older poplar stands, formed as a result of heart rot, provide vital shelter and nesting habitats for many smaller birds and mammals, especially in the Cascades (Nesom, 2003). Poplar seedlings and saplings provide a food source for grouse, quail, voles, rabbits, and deer (Silberhorn, 1999; USDA, 2002; Nesom, 2003). Poplar trees and branches are used by beavers as a food source and in the construction of dams and lodges (Silberhorn, 1999; USDA, 2002). Larger poplar stands are an important habitat, providing shelter and a food source for larger mammals such as porcupine, deer, and elk (Nesom, 2003).

Hybrid Poplar

Hybrids are produced when individuals of different, though often closely related, species are bred together. The goal is to produce offspring with heterosis. Heterosis is also known as hybrid vigor, and this is when the offspring produced exhibit traits, typically increased growth and survival rates, whose performance

exceeds that of either parent (Pearson, 2000; St. John, 2001). The cultivar used in this study is OP-367, a sterile male originally developed by a timber company in Maine (Pearson, 2000). Figure 4 shows 2 year old OP-367 clones from the clonal trials at the ERCO Tree Farm. The OP-367 cultivar, accession number 9076418, is a diploid hybrid resulting from a *P.deltoides* (female) x *P.nigra* (male) cross, and it is also referred to as the *P. x canadensis* clone or the Euroamerican hybrid (Han et al., 2000; St. John, 2001). This cultivar, also known as a clone, is produced through the vegetative reproduction, i.e. cuttings, of a single individual tree. Thus, all individual trees with the OP-367 identification came from one single tree and are genetically identical. Hybrids with a parentage similar to the OP-367, crosses of *P.deltoides* and *P.nigra*, are commonly referred to as a DN accession, and this is one of the three most commonly used parentages for developing hybrid poplars (St. John, 2001). The other parentages commonly used are the *P.trichocarpa* x *P.deltoides* (TD) accession and the *P.deltoides* x *P.maximowiczii* (DM) accessions (St. John, 2001). While many hybrids are produced via traditional means, the future production of poplar types will be the development of genetically engineered hybrids.

Figure 4. OP-367 Hybrid Poplar

Poplars have two characteristics that lend themselves to genetic manipulation: a relatively small genome and ease of transformation through bacteria. The poplar genome consists of nineteen chromosomes (Bradshaw 1996). This small size was one factor in black cottonwood being the first tree to have its genome completely sequenced. The amenability of poplars to transformation via *Agrobacterium* (Han et al., 1996) and the possibility of map-based cloning because of their small genomes make genetic engineering for pest resistance and other desirable traits feasible. Transgenic elite clones require limited field-testing and may be rapidly deployed without further breeding to stabilize transgenic traits (Bradshaw 1996).

One concern about hybrid poplar plantations and genetically engineered poplars is the possibility of these specialized, non-native trees hybridizing with native

species and harming the native gene pool. Narrowleaf (*P. angustifolia*) and Fremont (*P. fremontii*) species of poplar are common native poplar species in western United States riparian areas, and hybridization among these species with the introduced Lombardy poplar (*P. nigra*) does occur naturally. However, extensive planting of non-native poplar plantations in the western United States has had a limited effect upon the genetics and ecology of native riparian poplars even though these hybrid poplars may readily establish themselves (Heilman et al., 1995; U.S. Environmental Protection Agency, 1999). This lack of hybridization between native species and poplar plantations is due to a number of different physiological and ecological characteristics. These include: delayed flowering, tree longevity, vegetative persistence, existing wild stands of poplar, stringent habitat requirements, and the inability of both hybrid and native seedlings to establish under existing herbaceous vegetation (Strauss et al., 1999)

Poplar Uses

There are a number of different agricultural, commercial and environmental uses for poplar plantings. Outside of plantations used as a renewable wood source, other uses for poplar plantings include windbreaks, living snow fences, timberbelts, and riparian buffer strips (Kuhn and Rietveld, 1998; Brandle and Nickerson, 1996). Windbreaks provide shelter for livestock animals, such as cattle (Quam et al., 1994), and for slower growing crops, such as Christmas trees. A special type of windbreak, known as a living snow fence, is used as a low cost method for protecting roads and railways from blowing and drifting snow. They also provide shelter for birds and small mammals (Brandle and Nickerson, 1996). In addition, properly planned living

snow fences have the added benefit of increasing soil moisture by directing and trapping snow on fields. Timberbelts are strip plantings of trees that perform two tasks: they serve as conservation buffers in agricultural systems and generate supplemental income for the grower (Kuhn and Rietveld, 1998). These timberbelts also provide forested habitat, especially for birds and small mammals, in areas where most forests may have been replaced with field crops. Trees in this system are generally harvested via coppicing and are able to withstand up to four harvest cycles over a period of up to thirty years (Kuhn and Rietveld, 1998). Riparian buffer strips are essentially a special type of timberbelt planted along streams and rivers, and they are used for filtering agricultural runoff and for stream bank stabilization and restoration (National Agroforestry Center, 2000). Poplars planted as a riparian buffer strip act as a sponge, absorbing sediment, nutrients, chemicals, and animal wastes from agricultural runoff before the runoff has the opportunity to enter receiving water bodies (Kuhn and Rietveld, 1998; National Agroforestry Center, 2000). In addition, the high uptake rates for water and nitrate in poplar help these buffer strips to remove excess nutrients in shallow groundwater coming from adjacent farmland (Kuhn and Rietveld, 1998; U.S. Environmental Protection Agency, 1999). The ease of propagation and a fast growth rate of poplar plantings help provide conservation benefits faster (Kuhn et al., 1998). In stream bank stabilization and restoration, buffer strips help decrease scour under higher flows by anchoring soil with their root system, by slowing down floodwaters, by intercepting water borne debris, and by providing shade to help control water temperature (Kuhn and Rietveld, 1998; National Agroforestry Center, 2000; Nesom, 2003). Harvesting of biomass from the buffer

strip about every three years prevents excess accumulation of nutrients while also rejuvenating the buffer system itself (Kuhn and Rietveld, 1998). Other uses for poplar plantings that are either in development or are being used on smaller scales include the remediation of TNT contaminated soil and groundwater, as a tertiary treatment process for municipal wastewater, as an landfill cap to limit infiltration of water, and as a treatment for landfill leachate (Kuhn and Rietveld, 1998; National Agroforestry Center, 2000).

However, the most common large scale plantings of poplar are plantations. These plantations serve as a fiber source for the wood products industry. Different members and crosses of the genus *Populus* used in these plantings because they grow fast, they are easy to propagate, and the produce a high quality fiber (Stettler et al., 1996; St. John, 2001). The spacing used when planting cuttings is dependent on what the end product will be. Smaller tree spacing, such as 91 centimeters x 91 centimeters (3 feet x 3 feet) or 152 centimeters x 152 centimeters (5 feet x 5 feet), are used for biomass generation. Tree spacing up to 365 centimeters x 365 centimeters (12 feet x 12 feet) is necessary to produce the larger trees used for veneer or saw logs. With proper management, a typical ten year rotation results in a 6 meter (20 foot) saw log that has a 36-40 centimeter (14-16 inch) diameter (St. John, 2001). Assuming a good market for the size and type of poplar produced, a poplar plantation that invests $1114 per hectare ($450 per acre) per year may see a return of up to $24,752 per hectare ($10,000 per acre) after a 10 year growth cycle (Moore, 1997).

Products

In addition to biomass generation, there are many different uses for the wood produced in the different types of poplar plantings. These uses are largely dependent on the size of the tree. Smaller trees are used for landscaping mulch, oriented strand board, and as a fiber source for the wood products industry (National Agroforestry Center, 2000; Pearson, 2000). Larger trees are used for veneer, pallets, crates, lumber, building materials, and even for log homes (St. John, 2001). Other uses for poplar wood include the following: picture frames, toys, molding, cabinets, furniture, and caskets. One potential use for poplar biomass of all sizes is in the realm of energy production. Poplar wood contains high amounts of lignocelluloses, which is a possible starting material for the production of ethanol (Ugarte, 2003). Poplar biomass is also pelletized and co-fired with coal to produce electricity (Ugarte, 2003). The production of trees large enough to produce trim wood products is the goal for the trees grown at the ERCO plantation.

In addition to the size of the tree, the characteristics of the poplar wood itself are also important. The poplar wood materials prove easy to work with due to their dimensional stability, low weight and low rate of defects. Wood from hybrid poplars is generally superior to native poplars for a number of reasons. First, hybrids have a straighter bole than natives, resulting in more useable wood (St. John, 2001). Second, hybrids are generally easier to debark, resulting in quicker processing with less wear and tear on equipment (St. John, 2001). Finally, hybrids tend to have lighter colored heartwood, allowing for a more consistent application of paint and stains on finished poplar wood products (St. John, 2001; Nesom, 2003).

Remediation Characteristics

In addition to their wood products, poplar trees have characteristics that make them ideal candidates for phytoremediation purposes. These traits include fast growth rates, high nutrient uptake rates, and adaptability to a wide variety of environmental conditions. Depending on the species or hybrid, poplars may grow 1.5-2.4 meters (5-8 feet) per year. Growth in excess of 3.7 meters (12 feet) per year is possible under ideal conditions. Poplar trees have high rates of nitrogen uptake at 225-403 kilograms per hectare (200-360 lbs per acre) nitrogen per year (National Agroforestry Center, 2000). The daily water uptake of an individual poplar, dependent on the age of the tree and the season, is estimated at 4.5-303 liters (1.2-80 gallons) per tree per day (Ferro et al., 2001; Quinn et al., 2001). In the first year, the estimated water use for a poplar plantation is 0.103-0.144 meters per hectare (10-14 inches per acre) per year. This increases to 0.329-0.370 meters per hectare (32-36 inches per acre) per year by the fourth year (St. John, 2001). From a remediation perspective, poplar trees serve as an effective biological filter due to their high water demands. Under favorable conditions, a single poplar tree may extend their roots up to 7.6 meters (25 feet) below the surface in search of water. Combined with their uptake, poplars may be considered as a biological "pump" to control groundwater migration (Gatliff, 1994; Licht, 1995). The ability of poplars to grow under a wide range of environmental and physical conditions is also an important characteristic. Site conditions that may impair plant growth, such as salinity, pH, soil type, and climate, may be overcome by selecting the proper poplar species or clone. For example, while many poplars will

tolerate soil pH that ranges from 4.0 to 8.0, the OP-367 clone grows best on soils with a pH over 7.5 (St. John, 2001; USDA, 2002).

The combination of a high growth rate and a wide variety of acceptable conditions result in trees that are able to accumulate a large amount of biomass in a relatively short time span. These characteristics present an opportunity for biosolid utilization businesses to recover operational costs by selling tree products after harvest. A stand rotation of six to fifteen years should prevent deterioration of the stand and provide ample time for the trees to reach a marketable size (National Agroforestry Center, 2000). The combination of a high rate of growth, a high rate of nutrient uptake, water filtering ability and the possibility of an economic return from wood products make the hybrid poplar a good candidate for use in biosolids-based applications.

Nutrient Requirements

General Information

Plants require a wide variety of nutrients in differing concentrations for growth, reproduction, and many other biological processes. The amount of nutrients available for plant uptake and usage has a direct effect on the growth and survival of the plant. For crops like corn or cotton, providing the proper amount and type of fertilizer at the correct time is vital in order to maximize plant growth and crop yield. This timing is also applicable for tree plantations as well, especially in the first three years as the trees are becoming established in their environment. The elements carbon, hydrogen, and oxygen are used by plants as the main constituents of organic compounds. Carbon is supplied by the carbon dioxide in the atmosphere while

hydrogen and oxygen are supplied by water. The remaining nutrients needed by the plant for various compounds and functions are found in the soil. A mineral is described as an essential nutrient if it is required by the plant to complete its life cycle and is directly involved in plant metabolism (Arnon and Stout, 1939; Epstein, 1972). The main nutrients, known as macronutrients, utilized by plants in large qualities are nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur (Audesirk and Audesirk, 1999). Plants also utilize the elements iron, copper, manganese, zinc, boron, chlorine, molybdenum, and nickel in smaller quantities (Audesirk and Audesirk, 1999). These elements are known as micronutrients.

Macronutrients

The nutrients needed by plants in larger amounts are referred to as macronutrients. Nitrogen is often added for optimal plant growth. Phosphorus and potassium are also needed by the plant in large quantities but sufficient concentrations are generally available in the soil. The remaining macronutrients are absorbed from the soil and are usually found in sufficient concentrations. Generalized typical macronutrient concentrations and their roles in plants are summarized in Table 2 (Raven et al., 1981). Known as primary macronutrients, nitrogen, phosphorus, and potassium combine for about two-thirds of the total mineral nutrients found in plants. Calcium, magnesium, and sulfur are present in plants in smaller quantities and are known as secondary macronutrients.

Table 2. Typical Concentrations and Functions of Macronutrients in Plants

Nutrient	Concentration	Functions in Plant
C	44%	Organic Compounds
N	1%-4%	Amino Acids, Proteins, Nucleotides, Nucleic Acids, Chlorophyll
P	0.1%-0.8%	ATP/ADP, Nucleic Acids, Phospholipids
K	0.5%-6%	Enzymes, Amino Acids, Stomata Opening/Closing
Ca	0.2%-3.5%	Cell Walls, Cell Permeability
Mg	0.1%-0.8%	Chlorophyll, Enzyme Activator
S	0.05%-1%	Amino Acids, Proteins, Coenzyme A

The most important of the primary macronutrients is nitrogen, which is usually the most limiting nutrient affecting plant growth (Landis and van Steenis, 2003). Nitrogen helps promote green leaves and stems. Nitrogen is found in amino acids and is a key component of the chlorophyll molecule (Landis and van Steenis, 2003). Plants uptake nitrogen in two major forms: the nitrate and ammonium ions. Nitrate uptake and translocation occurs without the need for conversion into other forms (Landis and van Steenis, 2003). After uptake the ammonium ion must be converted by the roots into useable forms, such as amino acids, amides, or other compounds, before translocation occurs (Landis and van Steenis, 2003). Nitrogen is needed in the highest concentration in tissues where the plant is actively growing: young leaves, flowers, and root tips (Landis and van Steenis, 2003). Nitrogen is mobile in plant tissue and moves from older leaves to younger foliage as nitrogen supplies decrease (Landis and van Steenis, 2003). This mobility in plants makes initial nitrogen deficiency difficult to detect until after older leaves begin to change color from green to light green and finally to yellow as the chlorophyll molecules are broken down and nitrogen is moved to younger tissues (Landis and van Steenis, 2004a; Mahler, 2004). In addition, the presence of ammonium in the root zone may induce a calcium deficiency in the plant since both ions compete for binding sites in the roots (Landis and van Steenis, 2004a). Conversely, the presence of nitrate in the

root zone may actually enhance calcium uptake by the plant (Landis and van Steenis, 1996a). Foliar nitrogen levels at the lower end of the adequate range for plants results in slower growth and foliage that is lighter green in color (Landis and van Steenis, 2004a). Toxicity due to excess supplies of nitrogen is rarely seen due to the luxury consumption of nitrogen by plants (Landis and van Steenis, 2004a). However, excess nitrogen induces sulfur deficiencies and may delay the onset of dormancy, resulting in possible cold damage during winter months (Landis and van Steenis, 2004a). In addition, the efficiency of nitrogen use by plants decreases with increasing nitrogen fertilization (Landis and van Steenis, 2004a). This results in environmental issues due to excess nitrogen in runoff or with potential groundwater impacts from leaching.

Behind nitrogen, phosphorus is the second most important nutrient for plant growth and function. Functions of phosphorus within the plant include energy storage and release, as a major structural component of nucleic acids, as an enzyme regulator, and as a key component of cell membrane structure in the form of phospholipids (Landis and van Steenis, 2004b). Phosphorus is needed for the production of roots, flowers and fruits. A large portion of the available phosphorus in the soil is from organic matter. While phosphorus is very mobile and is translocated both up and down within the plant, the vast majority of the phosphorus in soil is unavailable to plants (Landis and van Steenis, 2004b). Under different pH conditions, magnesium, iron, aluminum, and calcium all bond to phosphorus, making the phosphorus non-labile and unavailable for plant use (Landis and van Steenis, 2004b). Except for the general appearance of stunted growth, there are no typical

visual or foliar symptoms for phosphorus deficiency (Landis and van Steenis, 2004b; Mahler, 2004). Thus, it is difficult to diagnose phosphorus deficiency.

Potassium is the last of the primary macronutrients and rivals nitrogen in terms of the amount taken up by plants and is the only macronutrient that is not a component of any plant structure (Landis, 2005). The roles of potassium in plants include the following: the opening and closing of stomata, water regulation, ATP production, the translocation of photosynthetic products, as an enzyme activator, cation-anion balance, and is presumed to play a key role in the frost hardening of plants (Landis, 2005). Potassium aids in flowering and fruiting, sturdiness, disease resistance, and stress resistance. Elemental potassium does not occur in nature due to its extremely reactive nature. Potassium is extremely mobile and easily transported between both the roots and the shoots, with young metabolically active tissues having a much higher concentration than either mature leaves or structural tissues (Landis, 2005). The bulk of potassium uptake occurs in the first half of the growing season (Lands, 2005). Potassium deficiency presents in older leaves first, with deficient plants having leaf margins that appear scorched (Mahler, 2004; Landis, 2005). This may occur when foliar potassium levels are less than 0.35% (Landis, 2005). Excess levels of potassium may interfere with the uptake of calcium and magnesium but appears to have no negative environmental impacts (Landis, 2005).

Calcium is the first of the secondary macronutrients. Mainly found in cell walls and plasma membranes, calcium aids in cell division, the strengthening of cell walls, the regulation of cell membrane permeability, and in toxin inhibition (Landis and van Steenis, 1996a). Calcium uptake is a function of soil solution availability, and

competition with potassium and ammonium decrease uptake rates (Landis and van Steenis, 1996a). Most calcium uptake takes place young at the root tips, and there is little translocation of calcium from older to younger tissues (Landis and van Steenis, 1996a). The lack of mobility of calcium within the plant results in deficiencies manifesting in the younger tissues of the plant, especially the growing tips of the plant (Mahler, 2004). However, the visual cues for calcium deficiency are difficult to detect before damage to the roots occurs (Landis and van Steenis, 1996a).

Then next secondary macronutrient is magnesium. Similar to iron in the hemoglobin molecule, magnesium plays a key role in photosynthesis as the only metallic constituent in the chlorophyll molecule (Landis and van Steenis, 1996b). In addition to its role as the central building block of chlorophyll, magnesium also plays a role in the following: the regulation of cellular pH and water resources, cation-anion balance, energy transfer, enzyme stabilization, the movement of phosphates, and seed formation (Landis and van Stennis, 1996b). Magnesium is mobile in plant and moves from older to younger tissues when deficient. Thus, deficiency symptoms appear first in older foliage. Visual symptoms of magnesium deficiencies in broadleaved hardwoods are foliar interveinal chlorosis, and the appearance of visual symptoms occurs when the magnesium deficiency is already severe (Landis and van Stennis, 1996b; Mahler, 2004). Excess potassium or ammonium fertilizers may induce a deficiency of magnesium, and excess levels of magnesium may induce deficiencies of calcium and potassium (Landis and van Stennis, 1996b).

The final secondary macronutrient is sulfur. Sulfur is supplied either through rainfall or through the weathering of rock. The main role of sulfur is as a key

component of proteins, coenzyme A, several vitamins, and as a structural component of the amino acids cysteine and methionine, (Landis and van Steenis, 1997). In addition, other roles of sulfur include the following: the regulation of ion transport across membranes, as a structural feature (sulfolipids) in cell membranes and polysaccharides, and it also contributes to the green color of the plant (Landis and van Steenis, 1997). Sulfur is taken up as the sulfate ion and must be converted into a usable form before incorporation into amino acids or proteins (Landis and van Steenis, 1997). Sulfur is fairly mobile in plants, with younger leaves in sulfur deficient plants turning yellow/yellow-green first followed by older foliage (Mahler, 2004). Plant tissue diagnostic is effective at determining sulfur deficiencies, especially when compared to healthy plants (Landis and van Steenis, 1997).

Micronutrients

The micronutrients are as follows: iron, copper, manganese, zinc, boron, chlorine, molybdenum, and nickel. Chlorine, molybdenum, and nickel were not evaluated in this study. These nutrients are needed in lesser amounts and are generally found in sufficient amounts in normal pH-balanced soils. However, a deficiency in any of these eight nutrients may affect the health of a tree. Generalized typical micronutrient concentrations and their roles in plants are summarized in Table 3 (Raven et al., 1981).

Table 3. Typical Concentrations and Functions of Micronutrients in Plants

Nutrient	Concentration	Functions in Plant
Fe	25-300 ppm	Chlorophyll Synthesis, Cytochromes, Nitrogenase
Cu	4-30 ppm	Enzyme Activator, Lignin Synthesis
Mn	15-800 ppm	Enzyme Activator, Nitrogen Metabolism
Zn	15-100 ppm	Enzyme Activator, Starch Formation
B	5-75 ppm	Influences Ca Utilization, Cell Wall Formation

The first micronutrient is iron. Iron plays a critical role in the manufacture of the chlorophyll molecule and in the functioning of photosynthesis (Landis and van Stennis, 1997). The uptake of iron is influenced by the presence and availability of other ions, particularly phosphorus, manganese, zinc, and calcium, in the root zone, and these interactions may continue within the plant, forming precipitates that are useless to the plant (Landis and van Stennis, 1997). Iron uptake is also negatively affected as soil pH increases, such as iron deficiency that is induced as a result of excess nitrate (Landis and van Stennis, 1997). Additionally, low levels of oxygen in the soil as a result of soil compaction or saturation also prevent plant uptake of iron (Landis and van Stennis, 1997). Iron is highly immobile in plants, and interveinal chlorosis in younger foliage is an early symptom of iron deficiency (Landis and van Stennis, 1997; Mahler, 2004)

The next micronutrient is copper. Copper plays roles in lignin synthesis, in enzymes in the oxidation-reduction processes, as a constituent of proteins, and helps with the efficiency of photosynthesis (Landis and van Stennis, 2000). Copper also plays a key role in carbohydrate and nitrogen metabolism. Copper uptake decreases with increasing soil pH and copper competes with aluminum for binding sites under low pH conditions (Landis and van Stennis, 2000). Increased levels of phosphorus and iron also decrease copper uptake (Landis and van Stennis, 2000). Copper is not mobile in plants, and deficiency symptoms appear in younger foliage first (Mahler, 2004). Symptoms of copper deficiency are variable, with foliar symptoms mimicking potassium deficiency (chlorosis and tip dieback) along with leaves that are blue-green (Landis and van Stennis, 2000). Low levels of deficiency result in a reduction of

photosynthetic activity and a decrease in plant turgor (Landis and van Stennis, 2000). Once a plant has become copper deficient it is unable to grow in search of it, and copper deficiency may induce nitrogen deficiency in nitrogen fixing plants (Landis and van Stennis, 2000). However, the preferential accumulation of copper in the roots makes the analysis of root tissue rather than foliar analysis a more accurate measure of copper deficiency or toxicity (Landis and van Stennis, 2000). Copper is considered toxic at concentrations of 20-30 ppm, and one side effect of copper toxicity is that it induces iron deficiency by shunting root growth (Landis and van Stennis, 2000). However, excess copper is rare except in areas treated with mine waste or sewage sludge or areas with repeated exposed to copper-based fungicides (Landis and van Stennis, 2000).

The next micronutrient is manganese. Manganese plays a role in the Hill Reaction in photosynthesis, as an enzyme catalyst, in carbohydrate synthesis, in lipid metabolism, as a structural component of ribosomes, in plant defense, and is important for root growth (Landis and van Steenis, 1998a). Manganese is not mobile in soil and it moves from the roots to the foliage once in the plant, with deficiency symptoms presenting in younger tissues first (Mahler, 2004; Altland, 2006). The main cause of manganese deficiency is high soil pH, and manganese uptake decreases with increasing soil pH or with high levels of available iron (Landis and van Steenis, 1998a; Altland, 2006). Symptoms of manganese deficiency are chlorosis of younger leaves, with foliar necrosis occurring under severe conditions (Landis and van Steenis, 1998a; Mahler, 2004). These symptoms are often confused with iron or zinc deficiency (Landis and van Steenis, 1998a). The appearance of foliar symptoms of

manganese deficiency indicates that a negative impact on plant growth has already occurred (Altland, 2006). While foliar diagnosis of manganese deficicieny is possible, a soil test is better at detecting deficiencies before plant growth is negatively affected (Altland, 2006). Manganese toxicity induces calcium and magnesium deficiencies (Landis and van Steenis, 1998a).

Following manganese, the next micronutrient is zinc. Zinc plays a role as a component in many enzyme systems, is essential for photosynthesis, assists in starch formation, and is important in rooting (Landis and van Steenis, 1998b). The availability of zinc in the soil decreases under high pH conditions and high phosphorus levels inhibit zinc uptake (Landis and van Steenis, 1998b). Zinc is relatively immobile in the soil and is immobile in the plant (Landis and van Steenis, 1998b). The lack of translocation from older to younger tissues leads to zinc deficiencies manifesting in younger tissues first (Landis and van Steenis, 1998b; Mahler, 2004). Zinc uptake decreases with increased soil pH, and high levels of available phosphorus or iron adversely affect zinc uptake (Landis and van Steenis, 1998b). Zinc toxicity is rare except in areas where biosolids are applied as a soil amendment (Landis and van Steenis, 1998b).

The final micronutrient is boron. Boron, along with chlorine, is the only micronutrient that is not a metal. Boron forms stable covalent bonds similar to carbon and does not occur in nature in a free state (Landis and van Steenis, 2004c). Boron plays a role in cell division, cell wall formation, amino acid and protein synthesis, carbohydrate metabolism, development and growth of new cells in the root tips and at the end of stems, and the translocation of nitrogen, phosphorus, sugars, and

starches (Landis and van Steenis, 2004c). The amount of boron needed by the plant depends on plant species, age, and climate (Landis and van Steenis, 2004c). Plant availability of boron decreases with increasing pH and is also affected by the amount of water in or moving through the soil profile (Landis and van Steenis, 2004c). Since boron is present in soils as an anion it is easily leached out of the root zone under high rainfall events (Landis and van Steenis, 2004c). Conversely, passive uptake of boron through plant roots tips is limited under drought or other conditions that restrict root activity or decrease the mass flow of water to the plant (Landis and van Steenis, 2004c). Similar to calcium, boron is immobile once assimilated by the plant, making translocation from older to younger tissues impossible once boron becomes deficient (Landis and van Steenis, 2004c; Altland, 2006). Thus, deficiency symptoms present in younger tissues first. Symptoms of boron deficiency are a deformed growing tip (Mahler, 2004). Boron toxicity results in chlorosis or necrosis of the terminal bud or tips of mature leaves (Landis and van Steenis, 2004c). The use of biosolids as a soil amendment may increase the amount of boron in the soil to toxic levels (Landis and van Steenis, 2004c).

Water Stress

General Information

Water is a vital component for all forms of life. More so than nutrients, water availability is often the limiting factor that determines the performance of nursery plants (Landis et al., 2005). In plants, water plays a key role in the movement of nutrients throughout the plant. As water evaporates from the surfaces of the leaves, it pulls water upwards from the root system, thus transporting nutrients and other

solutes to the above ground components of the plant (Audesirk and Audesirk, 1999). When water is in short supply, plants become stressed as amount of water taken up by the roots is unable to keep up with the rate of evaporation of water from the leaves. As a result, the leaves of the plant begin to wilt as the amount of water present within the leaf tissue decreases. Water stress is typically well developed and negatively affecting the plant by the time it is detected, with visual detection of water stress indicating high levels of water stress. The amount of water stress, both chronic and acute, that a plant is able to withstand before dying varies by plant species, location, and environmental conditions (Eitel et al., 2006).

Implications for Poplar

Plantations of *Populus* in the Eastern Cascades region of North America are often subject to water stress and irrigation is often required by mid-summer (Zsuffa et al., 1996). If undetected, water stress represents a major economic loss due to poorly timed irrigation, decreased growth rates, increased mortality, increased susceptibility to pathogens or infestations, and an increase in time beyond the typical five or six year rotation needed to produce marketable trees (Zsuffa et al., 1996; Ragazzi et al., 1999; Eitel et al., 2006). The timing of irrigation is typically based on measurements of soil water potential. This method is time consuming and difficult to collect and interpret on a plantation scale basis. As a result, there are fewer samples taken, and the lack of sampling may overlook localized water stress events (Eitel et al., 2006). Thus, other methods are needed that will accurately characterize water stress levels.

Methods of Measuring Leaf Water Potential

In addition to measuring soil water potential there are a few other methods of determining plant water stress levels. One conventional method that is commonly used to determine plant water stress is by measuring leaf water potential (Turner, 1981; Eitel et al., 2006). Leaf water potential is the amount of pressure needed to force water out of the leaf through the petiole (Cleary and Zaerr, 1980). This pressure is equivalent to the capillary pressure of the plant tissues within the leaf, and the more pressure required to force water out corresponds to a higher level of water stress (Roth and Goyne, 2004). This method utilizes the Scholander pressure bomb for determining pressure. Figure 5 shows the experimental setup for the Scholander pressure bomb, manufactured by PMS Industries, used in this study. One drawback to this method is that it is difficult to determine exactly when bubbles form in the sap on the tip of the petiole. An incorrect observation of these bubbles directly affects the determination of the pressure required (Ritchie and Hinckley, 1975).

Figure 5. Water Stress Testing Apparatus

Newer methods of water stress detection and measurement revolve around the use of remote sensing. The use of the thermal part of the spectrum is the primary means of remote sensing water stress, and the relationship between water stress and thermal readings were developed into the crop water stress index (Barnes et al., 2000). The use of reflectance readings and their associated indices represents a non-thermal method of determining plant water stress levels (Barnes et al., 2000). The study of the impact of water content on spectral response at the laboratory scale shows that there is a strong correlation between water content and the shortwave infrared reflectance (Toomey and Vierling, 2005). However, each of these methods has drawbacks. One weakness of the crop water stress index is that when the index

begins to respond to changes in soil moisture levels the amount of moisture in the soil has already dropped by almost 50% (Barnes et al., 2000). A key drawback to reflectance based indices is their dependence on chlorophyll. Many nutrient deficiencies affect the level of chlorophyll found in the leaves, and this makes the interpretation of many simple indices based on a single nutrient difficult (Barnes et al., 2000). In addition, any deficiency that changes the canopy density also affects indices based on the red and near-infrared wavelengths (Barnes et al., 2000). Another key consideration affecting the application of remote sensing is the ability of satellites to measure reflectance at the major water bands located at 1450 nm and 1900 nm is often limited by interference from the Earth's atmosphere (Dallon and Bugbee, 2003).

Foliar Analysis

General Information

Determining plant nutrient deficiencies is accomplished through the testing of either the soil in which the plant is growing or through the analysis of plant tissues. Soil tests provide information about the level of nutrients available to the plant for uptake while plant tissue tests provide information about the amount of nutrients that the plant has actually utilized (Brockley, 2001). Due to the ease of collection, leaves are the most common type of plant tissue that is typically analyzed for nutrient content. Compared to soil tests, the main reason for the use of foliar analysis is that the leaves are a better indicator of plant nutrient status than soil due to the difficulty of collecting a soil sample that is representative of nutrient availability throughout the entire root zone of the plant (Leyton, 1957; Brockley, 2001; Zatylny and St-Pierre, 2005). The usefulness of foliar analysis is that a deficiency in a particular nutrient is

reflected in the concentration of that nutrient in foliage (Leyton, 1957). Additionally, foliar analysis is both a reliable and a cost-effective method of determining nutrient deficiencies (Brockley, 2001). Mobile nutrients, such as N, P, K, Mg, Cl, and Mo, are typically found with higher concentrations in younger tissues that are actively growing (Landis et al., 2005). Thus, deficiency symptoms for these nutrients generally appear first in older tissues. Conversely, nutrients that are immobile within the plant, such as Ca, S, B, Fe, Mn, Zn, and Cu, tend to have higher concentrations in older tissues (Landis et al., 2005). Hence, deficiency symptoms for these nutrients initially manifest on juvenile tissues first.

Guidelines

Most of the guidelines for foliar analysis are based on end-of-the-year values once growth has stopped (Landis et al., 2005). This is because nutrient concentrations change throughout the course of the growing season, with nutrient concentrations, especially for the macronutrients N, P, and K, becoming relatively stable in late summer (Brockley, 2001; Luyssaert et al., 2002). Since the guidelines for foliar testing are primarily based on N and P, the stability of other nutrients at the end of the year, especially secondary macronutrients and the micronutrients, is not fully understood. The leaves selected for testing should be fully developed and collected well before the onset of senescence (Leyton, 1957; Luyssaert et al., 2002).

Limitations

It is also important to keep in mind that there are a number of limitations of foliar analysis. First, plant growth is influenced by a number of variables other than the availability of a single nutrient (Leyton, 1957). For example, soil pH affects

nutrient imbalances by making certain minerals more toxic while limiting the availability of other minerals to the plant even if these minerals are present at adequate levels in the soil (Callan, 1998). Second, foliar concentrations of certain nutrients are affected by the process of luxury consumption (Leyton, 1957). Third, foliar concentrations are affected by the position of the leaf within the tree crown and the age of the leaf itself (Brockley, 2001). Fourth, leaves that are in the shade have higher foliar concentrations of N, P, Mg, and Ca than leaves that are predominantly in the sun (Luyssaert et al., 2002). Fifth, the availability, uptake, and foliar concentration of N decrease during periods of drought (Luyssaert et al., 2002). Next, foliar symptoms are often a combination of multiple nutrient deficiencies, and by the time some of these deficiencies manifest, the damage to the plant is already done (Malhotra and Blauel, 1980; Callan, 1998). Finally, it is important to keep costs, both financial and labor related, in perspective. The cost of testing for all of the macronutrients and micronutrients by A & L Eastern Labs is around $22.00 per sample. This does not include additional material costs, shipping costs, and labor costs, running at about $10.00 per hour per laborer, incurred while collecting, bagging, and shipping the leaves to the laboratory for foliar analysis.

Reflectance

General Comments

Reflectance is a measurement of the amount of light energy reflected by a leaf. Remote sensing in the 300 to 1100 nm wavelength range (visible and near infrared) is based on the principle that reflectance properties of vegetation are a function of changes in light interception and utilization by the plant (Carter, 1998;

Jensen, 2000). The use of spectral data represents a powerful analysis tool due to the potential for narrow wavebands to correlate with specific plant variables (Hansen and Schjoerring, 2003). A study by Read et al. (2002) showed that at the canopy level, nitrogen stress in cotton correlates with an increased reflectance at the 695 nm wavelength and a decreased reflectance at the 410 nm wavelength. Thus it may be possible to derive a relationship between changes in leaf reflectance and changes in nutrient levels. A study by Fridgen and Varco (2004) evaluated nitrogen concentration and potassium availability in cotton. A key finding in this study was that is was indeed possible to detect distinct nutrient deficiencies using the partial least squares method to analyze leaf reflectance data in the visible and near infrared regions as long as there were no other nutrients deficient. Studies by Chua et al. (2003) and Bronson et al. (2003) on cotton showed that indices developed from reflectance data, specifically reflectance in the green and red wavebands, were able to predict cotton crop in-season nitrogen fertilizer requirements. Another study used reflectance data to model rice growth (Yang and Chen, 2004). This study found that growth parameters, such as plant height and leaf area index, were negatively correlated with reflectance in the visible region but were positively correlated with reflectance in the near infrared region. Additionally, relationships between the reflectance of wetland species and different water quality parameters have been determined (Tilley et al., 2003). Specifically, changes in total ammonia concentrations in the water were detected utilizing the photochemical reflectance and red-edge reflectance indices derived from broad-leaved cattail reflectance data.

Project Implications

The implications for this project are as follows: foliar nutrient concentrations change in response to nutrient availability. These changes in foliar nutrient concentrations result in measurable changes in the spectral reflectance of hybrid poplar leaves. The changes in leaf reflectance make it possible to model leaf foliar nutrient concentrations based on reflectance data.

Objectives

The objectives for this project are as follows:

Objective 1: Measure the reflectance spectrum wavelength of the hybrid poplar clone OP-367 for trees that range from zero to seven years in age.

Objective 2: Determine if the reflectance spectrum of the leaves correlates, either through direct evaluation of wavelengths or through regression analysis, with the macronutrient concentrations, micronutrient concentrations, and water stress levels measured in the leaves.

Objective 3: Develop models that predict foliar nutrient concentrations and leaf water potential levels based on reflectance data.

Objective 4: Evaluate the use and effectiveness of models based on reflectance data as a nutrient management or water management tool for hybrid poplar OP-367.

Materials and Methods

Site Description

The study site for this project is located on the ERCO Beneficial Reuse Tree Farm in Brandywine, Maryland. This is a privately held farm and it is located in southern Prince George's County on what was once a sand and gravel quarry. It is located in the coastal plains physiographic region approximately 32 kilometers (20 miles) east of the piedmont physiographic region. The site description is from Wilson and Fleck (1990) and Tompkins (1983), and the site consists of remnants of the Pliocene Upland Deposits overlaying the lower Miocene Calvert Formation. The Upland Deposits consist of orange-tan, silty, fine to very coarse sands and gravels, and yellowish to orange, silty clays while the Calvert is a light to medium, olive gray to olive green, micaceous, clayey silt (Buswell, 2006). The majority of the Upland Deposits were removed during the sand and gravel mining operations.

ERCO Operations

Once mining operations ceased, the site was re-graded and divided into nine sections as seen in Figure 6. Each section is roughly 4.04 hectares (10 acres) in size. Biosolids are applied to one section per year.

Biosolids incorporation begins each day when a section of trench is dug prior to the delivery of biosolids. Biosolids are transported from the Blue Plains facility and dumped into the waiting trenches. These trenches are then covered within one hour of delivery to minimize odor and to keep the biosolids in an anaerobic state. This process is repeated until biosolids have been applied to the entire section. The

section is then re-graded and trees are planted in the spring on ten foot centers to allow for the operation of heavy equipment between the rows of trees. Biosolid application operations continue year round, and the planting rotation results in a maximum of two sections without trees at any one point in time.

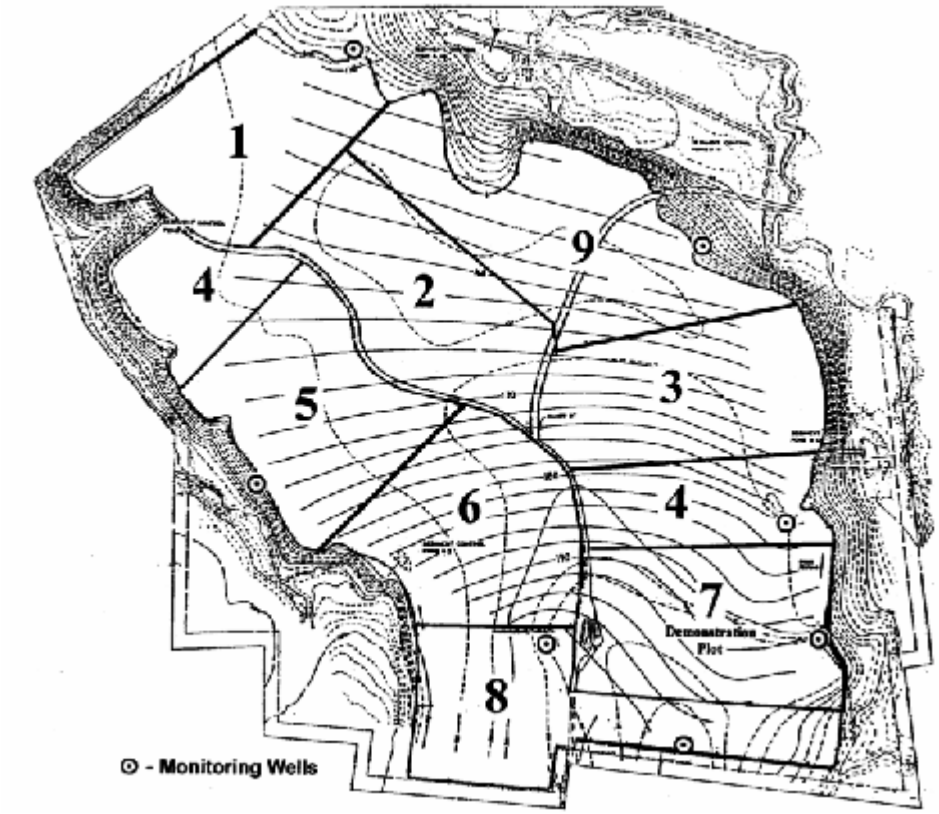

Figure 6. Overview of the Different Sections at the ERCO Tree Farm

Experimental Design

Farm operations divided the study area into nine sections approximately ten acres in size. In addition, all of the trees in a given section were planted at the same time. The ages of the trees in the different sections range from those that were completing their first growing season, known as rising ones, through trees completing their seventh growing season, representing trees planted in consecutive years (1999-

2007). Each section was planted with the same type of hybrid poplar, the OP-367 clone. This clone was chosen based on the results of a clonal field trial and was also based on growth rate, cold tolerance, and soil pH tolerance. In addition to being planted with the OP-367 clone, each section also had the same one-time biosolids application rate of 171 dry tons per acre, the same planting density of 430 cuttings per acre, and the same tree spacing of 10 foot centers (Kays et al., 2000). Trees within each section were randomly selected for sampling. The outermost trees along the borders of each section or along the access roads were excluded in order to avoid potential edge effects. Five sections, ages 1 to 5, with ten trees per section were sampled in 2004. The sampling for 2005 consisted of four sections, ages 2 to 5, with four trees per section. The age 1 trees were not sampled due to extreme deer browse damage. Inclement weather combined with equipment problems prevented the collection of leaves from the 6 year old trees. The number of trees sampled per section decreased based on a combination of cost considerations, in order to match the current sampling regimen employed by ERCO and in accordance with the UN/ECE-EC (1998) sampling guidelines (Luyssaert et al., 2002). Eight sections, ages 0 to 7, with five trees per section were sampled in 2006. Finally, five sections, with trees age 2 and ages 4 to 7, with five trees per section were sampled in 2007. Due to a combination of drought and deer damage, the trees of ages 0, 1 and 3 were not sampled in 2007. The different ages of trees for each section for each sample year are summarized in Table 4.

Table 4. Tree Age by Section 2004-2007

Section	2004	2005	2006	2007
1	4	5	6	7
2	*	*	2	*
3	*	*	*	*
4	*	*	1	2
5	5	*	7	*
6	3	4	5	6
7	1	2	3	4
8	2	3	4	5
9	*	*	0	*

* indicates that no foliar samples were taken

Sampling

Sample Collection

Poplar tree leaves were collected at the end of the growing season (late August or early September) before the onset of senescence. During this time period foliar nutrient levels, particularly for nitrogen and phosphorus, are at their most stable levels. Towards the end of September senescence begins and foliar nutrient levels change as mobile nutrients are moved out of the leaves into other plant tissues for storage. Trees were randomly selected for sampling, and a leaf was selected from the terminal leader. The leaves chosen for sampling were the first mature leaves, generally located between the fifth and seventh leaf down from the tip of the main leader. Each leaf selected was mature, completely unfurled, and free of tears or insect damage. When necessary, a 12 gauge shotgun using a heavy dove load was used to shoot off the topmost section of the main leader in order to collect a leaf sample from the higher trees. Leaves were collected from five trees within one section at a time, placed in a labeled brown paper bag, and brought to a centrally located sampling station for testing. Only one section was sampled at time in order to minimize the

potential impact that removing the leaf from the stem may have on leaf reflectance and leaf water potential measurements.

Leaf Reflectance

Reflectance measurements were taken for each of the trees sampled within each section. An ASD FieldSpec Handheld spectroradiometer (Analytical Spectral Devices Inc.; Boulder, CO) was used to measure reflectance wavelengths from 325 to 1075 nm by scanning 1.6 nm intervals. The spectroradiometer utilized a 1-degree foreoptic that narrowed the field of view of the instrument, helping to eliminate background interference. Prior to taking measurements, the spectroradiometer was calibrated using a Spectralon white panel (Labsphere, Inc.; North Sutton, NH). Reflectance from the white panel corresponded to 100% reflectance, and the sample percent reflectance was determined by dividing the observed reflectance by the reflectance of the white panel. White panel reflectance was checked and recalibrated (making adjustments if necessary) prior to evaluating each sample. Once the white panel calibration was completed, the reflectance of the sample leaf was measured and recorded. For each leaf, reflectance was measured ten times over a 36-96 millisecond increment, based on sunlight intensity, in order to generate an average reflectance value for the leaf. Measurements were taken from late morning to early afternoon (10:00 AM until 3:00 PM Eastern Standard Time) under incident solar radiation. Additionally, reflectance measurements were taken only on days with little or no cloud cover in order to minimize the impact of solar variation on leaf reflectance. This process was repeated until all the selected trees within each section were sampled. This entire process was repeated for each of the other sections sampled.

After the spectral data collection was complete, the leaves were then tested for leaf water potential.

Leaf Water Potential

Leaf water potential levels were measured using a pressure chamber PMS Instruments Model 600 (Soilmoisture Equipment Corp.; Santa Barbara, CA). This step utilized the same sample leaves used in gathering reflectance data. The stems of the sample leaves were trimmed with a razor such that the cut was clean and flat. This was done so that it was easier to see the water bubbles forced out of the petiole as pressure was applied to the chamber. Sample leaves were placed in the chamber, per PMS Instrument guidelines, with the stem of the leaf protruding through the gasket of the compression gland. The compression gland was tightened until the gasket grasped the sample, preventing gas from leaking out of the chamber along the leaf stem. The compression gland was inserted into the pressure chamber and inert gas (nitrogen) was slowly introduced into the pressure chamber. Once the pressure within the chamber was high enough to force water bubbles from the tip of the sample petiole, the gas flow was stopped and the pressure was recorded. This process was repeated until all of the selected trees within each section were tested. The entire process, leaf reflectance and leaf water potential, was repeated for each of the other sections. Once the leaf water potential sampling was complete, the leaves were placed back into the brown paper bags labeled with the sample name and put into cold storage until foliar nutrient analysis testing was performed.

Foliar Analysis

2004

The 2004 leaf samples were taken to the Research Environmental Analysis Lab at the University of Maryland for foliar analysis. Each sample was oven dried, ground, and tested for the following: %C, %H, %N, %P, %K, %Ca, %Mg, %S, ppm of Mn, ppm of Zn, ppm of Cu, and ppm of Fe. The combustion furnace technique was used to determining C, H, and N concentrations. All other nutrient concentrations were determined using acid digestions and ICP emission spectroscopy.

2005-2007

Due to the closing of the Research Environmental Analysis Lab at the University of Maryland, the leaf samples for the years 2005 through 2007 were analyzed by A&L Eastern Labs (Richmond, VA). Each sample was oven dried, ground, and tested for the following: %C, %N, %P, %K, %Ca, %Mg, %S, %Na, ppm of Mn, ppm of Zn, ppm of Cu, ppm of Fe, ppm of Al, and ppm of B. The combustion furnace technique was used to determining C and N concentrations. All other nutrient concentrations were determined using acid digestions and ICP emission spectroscopy. Samples were not tested for %H. Additionally, the samples for 2006 and 2007 were not tested for %C.

Data Analysis

The values for foliar concentrations were entered into a spreadsheet for further analysis. Mean values for nutrient concentrations and leaf water potential data were determined for each of the different age groups. Foliar nutrient concentration results

were compared to published guidelines found in Hansen (1994), Hansen et al. (1988), and summarized in Brown (1999) and Zabek (2001). Results were also compared to nutrient concentrations reported by Sylvis Environmental (New Westminster, Canada) to ERCO on a yearly basis for determining when tree harvesting and biosolids reapplication is applicable.

Statistical tests utilizing the GLM and MIXED procedures in SAS (SAS Institute, Inc.; Cary, NC) were used to determine the effect of age on foliar nutrient concentrations. Assumptions required for the use of ANOVA were checked using Levene's test for the homogeneity of variances (HOV), the graphs of the distributions of the residuals and predicted values, skew values, kurtosis values and the Shapiro-Wilk test statistic. The Welch's test was also utilized when needed. The key value for Levene's HOV test was a p-value greater than 0.05, indicative of equal variances between the different groups may be assumed. The graphs of the distributions of the residuals and predicted values were interpreted in order to determine the type of data transformation necessary for normal distribution. The absolute values for skew (asymmetry) and kurtosis (peakedness/flatness) was compared to table values. The null hypothesis of no skew/peakedness was rejected if the absolute value of the test statistic was greater than that of the table value. The Shapiro-Wilk test statistic was used to determine if the samples came from a normally distributed population. Values for the Shapiro-Wilk test greater than 0.95 indicate that the sample population is normally distributed. The Tukey-Kramer method was used in SAS for means comparison. This is a conservative method that keeps that Type I error rate (false positive) as low as possible by controlling the experimentwise error rate (Ott and

Longnecker, 2001). All tests were performed at the significance level of α equal to 0.05. Each year was analyzed individually to determine any significant differences between tree ages within a given year and as a pooled data set in order to evaluate the significance of foliar nutrient trends over the course of the study.

Regression analysis with the partial least squares (PLS) method was used to determine correlation coefficients between reflectance data and nutrient concentrations and reflectance data and leaf water potential. While principal components analysis (PCA) evaluates one data matrix, either the X or Y, to make a predictive model, the PLS regression method evaluates X and Y simultaneously in making a predictive model. This allows for the use of multiple independent variables to predict the response of one dependent variable (Garthwaite, 1994). Thus, it is possible to use spectral reflectance data in the prediction of nutrient and leaf water potential measurements by developing a set of components which are relevant to both sets of variables. These components contain the information in the independent variables that is used to predict the variability of the dependent variables (Garthwaite, 1994). The general equation in matrix form for the PLS linear regression model is:

$$Y = XB + E \qquad \text{(Equation 1)}$$

where Y is the variable response matrix, X is the variable predictor matrix, B is the regression coefficient matrix and E is a noise term with the same dimensions as Y. A factor score matrix T is computed using:

$$T = XW \qquad \text{(Equation 2)}$$

where W is the weight matrix for X. T and Y are used to generate the linear regression:

$$Y = TQ + E \qquad \text{(Equation 3)}$$

where Q is the matrix of regression coefficients (or loadings or weights for Y). Substituting Equation 2 into Equation 3 and comparing the result to Equation 1 shows that partial least squares regression coefficients B of Y on X are given by:

$$B = WQ \qquad \text{(Equation 4).}$$

Finally, a linear regression of X is generated using:

$$X = TP + F \qquad \text{(Equation 5)}$$

where P is the factor loading matrix and F is the unexplained part of the X scores. Applied to this study, the independent (or predictor or factor) variables used in the PLS regression analysis are from the spectral data set and the dependent (or response) variables are from the foliar nutrient and leaf water potential datasets.

PLS regression analysis was performed using Unscrambler 9.1 software (Camo Inc.; Oslo, Norway). Full cross-validation models, i.e. the leave-one-out model, were constructed on the pooled nutrient and spectral data from 2004-2007. The Martens uncertainty test was utilized in the full cross-validation model in determining the wavelengths (predictors) that had a significant impact on the model. The Martens uncertainty test compares the significance of the regression coefficient B generated for each of the samples in the sub-model to the overall regression coefficient B for each sample in the completed model. Test set models were constructed using 2004-2006 data for calibration and 2007 data for validation. The calibration stage is when the model is fitted to the available data. Once this is complete, the data variation is expressed as the sum of the modeled part (structure) and a residual part (error). The validation stage is when X-values of the samples not

used in the calibration stage are plugged into the model in order to generate observed Y-values. The observed Y-values are compared to the actual Y-values, used to compute the prediction residual, and this prediction residual is then used to determine the root mean squared error of prediction (RMSEP) for the validation stage.

The wavelengths used in the PLS models were either truncated (400-900 nm) or the visible and red edge (350-760) wavelengths. Wavelengths were transformed using Savitzky-Golay smoothing and Savitzky-Golay 1^{st} and 2^{nd} derivatives, both individually and in combination with one another. Savitzky-Golay smoothing replaces original values with values with regular variation by fitting a polynomial to each successive curve segment. Savitzky-Golay 1^{st} and 2^{nd} derivatives calculate the derivative of the wavelength data and add a smoothing function. The analysis used was the PLS2 model utilizing wavelength data to predict multiple dependent variables foliar nutrient concentrations simultaneously.

Results and Discussion

Foliar Nutrient Data

General Comments

Foliar samples were collected 2004-2007. Foliar hydrogen and leaf water potential were only analyzed in 2004. Foliar carbon was analyzed in 2004 and 2005 and was discontinued after 2005. Foliar sodium, boron, and aluminum were analyzed in 2005-2007. All other nutrients were analyzed 2004-2007. Each nutrient was evaluated on both a year by year basis and pooled together in order to evaluate the effects of age, the impact of weather and for determining general trends in foliar nutrient concentrations over the course of the study. Foliar nutrient results that were unremarkable were grouped together by year in Appendix 1. Mean foliar nutrient concentrations for 2004-2007 are summarized in Table 5.

Table 5. Mean Foliar Concentrations 2004-2007

Nutrient	Mean	Std. Error	n
%C	47.82	0.08	66
%H	5.33	0.04	50
% N	3.10	0.04	131
% P	0.27	0.01	131
% K	1.80	0.07	131
% Ca	1.26	0.04	131
% Mg	0.33	0.01	131
% S	0.36	0.01	131
Fe ppm	113.16	4.49	131
Cu ppm	9.16	0.26	131
Mn ppm	159.18	11.48	131
Zn ppm	67.13	2.41	131
B ppm	40.22	1.71	81
% Na	0.01	0.00	81
Al ppm	30.40	2.98	81

2004

Trees of ages 1 through 5 were sampled in 2004. The results for foliar carbon, sulfur and copper were unremarkable and are located in Appendix 1.

Hydrogen

The results of the foliar testing for hydrogen are found in Table 6. For 2004, the Levene's HOV test statistic on untransformed data had a p-value of 0.0039. Since this value is less than 0.05, the variances within the different ages of trees are unequal (Levene, 1960; Ott and Longnecker, 2001). Further transformations did not result in any improvement of the Levene's HOV test statistic p-value. However, both the graphs of the residuals and the value of the Shapiro-Wilk test statistic of 0.9807 on the untransformed data indicate that transformations before running an ANOVA were not necessary. The ANOVA results using a Tukey adjustment are presented in Table 6. These results indicate that the general trend is an increase in foliar hydrogen levels with tree age, with significantly higher foliar hydrogen levels in older trees ages 4 and 5 compared to younger trees. Since hydrogen is taken up by the plant directly through air and water, the fact that older plants have higher levels of foliar hydrogen is probably just an indicator of the larger root mass and surface areas available for transport processes in older trees. Foliar hydrogen in these poplars is marginally lower than the generalized ideal plant foliar hydrogen concentration of 6% (Raven et al., 1981). Whether this difference is meaningful or not is undeterminable at this time due to the lack of data for foliar hydrogen in poplar.

Table 6. 2004 ANOVA for Foliar Hydrogen (%)

Age	Mean	SD	N	Tukey
1	5.04	0.19	10	A
2	5.18	0.11	10	A
3	5.19	0.09	10	A
4	5.64	0.10	10	B
5	5.62	0.06	10	B

Means with the same letter are not significantly different

Nitrogen

The results of the foliar nitrogen testing are found in Table 7. Evaluation of the 2004 foliar nitrogen data indicated that data transformations were not necessary. The results of an ANOVA with Tukey adjustment on untransformed data are presented in Table 7. The ANOVA results indicated that the only significant difference between the different ages is that the age 1 trees have significantly lower foliar nitrogen concentrations than all other ages of trees. The general overall trend for the 2004 foliar nitrogen is an increase in concentrations with increasing age. This is most likely a reflection of the development of the root structure as the tree ages, thus allowing for increased nutrient uptake. One possible explanation for the lower foliar nitrogen values for the age 1 trees is that the roots of the trees may not penetrate the overburden and reach the depth where the biosolids are incorporated in the soil profile until the end of their second year of growth. Additionally, all ages show foliar nitrogen levels lower than the 3.5% benchmark required for fast growth.

Table 7. 2004 ANOVA for Foliar Nitrogen (%)

Age	Mean	SD	N	Tukey
1	2.58	0.38	10	A
2	3.08	0.32	10	B
3	3.08	0.21	10	B
4	3.22	0.27	10	B
5	3.28	0.20	10	B

Means with the same letter are not significantly different

Phosphorus

The results of the testing for foliar phosphorus are found in Table 8. Log transformation was performed on the foliar phosphorus data from 2004 based on the decrease in values for skew and kurtosis and the improvement in the value of the Shapiro-Wilk test statistic. The results of an ANOVA with Tukey adjustment on the log transformed data are presented in Table 8. The general trend for the 2004 data appears to be an increase in foliar phosphorus concentrations with increasing age. The age 1 trees have significantly lower foliar phosphorus levels than ages 3, 4, and 5. The age 2 trees have significantly lower foliar phosphorus levels than the age 4 and age 5 trees. The differences between the younger and older trees are most likely a result of the older trees having a more established root system, especially around the biosolids, that is able to uptake more phosphorus from both the soil and from the biosolids. Only the age 4 and age 5 trees show mean foliar phosphorus levels above the 0.30% ideal for fast growth.

Table 8. 2004 ANOVA for Foliar Phosphorus (%)

Age	Mean	SD	N	Tukey
1	0.22	0.05	10	A
2	0.25	0.04	10	A, B
3	0.27	0.04	10	B, C
4	0.33	0.07	10	C, D
5	0.36	0.04	10	D

Means with the same letter are not significantly different

Potassium

Foliar potassium results are found in Table 9. The 2004 foliar potassium data were log transformed based on the improvement in the values for the Shapiro-Wilk test statistic, kurtosis and skew. Table 9 contains the results of an ANOVA with Tukey adjustment on the log transformed data. The general trend for 2004 was an

increase in foliar potassium levels with age, peaking in the age 4 trees. The age 4 trees had significantly higher levels of foliar potassium than all other ages, while age 1 trees had significantly lower levels of foliar potassium than all other ages except for age 2. The differences in foliar potassium values between the younger and older trees are most likely a result of the older trees having a more developed root system that increases access to nutrients, especially to the nutrients found in the biosolids.

Table 9. 2004 ANOVA for Foliar Potassium (%)

Age	Mean	SD	N	Tukey
1	1.23	0.21	10	A
2	1.33	0.16	10	A, B
3	1.58	0.19	10	B, C
4	2.23	0.61	10	D
5	1.70	0.19	10	C

Means with the same letter are not significantly different

Calcium

Foliar testing results for calcium are found in Table 10. Log transformation was performed on the foliar calcium data from 2004 based on the graphs of the residuals and the improvement in the value of the Shapiro-Wilk test statistic. The results of an ANOVA with Tukey adjustment on the log transformed data are presented in Table 10. While the general trend for the 2004 data appears to be a decrease in foliar calcium concentrations with age, the only significant difference was between the trees with higher foliar concentrations, ages 1 and 2, and the age 4 trees which had the lowest levels of foliar calcium.

Table 10. 2004 ANOVA for Foliar Calcium (%)

Age	Mean	SD	N	Tukey
1	1.41	0.44	10	A
2	1.23	0.28	10	A
3	1.05	0.19	10	A, B
4	0.91	0.15	10	B
5	1.11	0.21	10	A, B

Means with the same letter are not significantly different

Magnesium

The results for foliar magnesium are found in Table 11. Log transforming the foliar magnesium data for 2004 improved the skew found in the graphs of the boxplot and the value of the Shapiro-Wilk test statistic while maintaining a significant p-value for Levene's HOV test. The results of an ANOVA with Tukey adjustment on the log transformed data are presented in Table 11. These results indicate that the age 1 trees have significantly higher levels of foliar magnesium than trees aged 3, 4, and 5. Additionally, the age 4 have significantly lower levels of foliar magnesium than all other tree ages except for age 5. The general trend for the 2004 foliar magnesium data indicates a decrease in foliar magnesium concentrations with age.

Table 11. 2004 ANOVA for Foliar Magnesium (%)

Age	Mean	SD	N	Tukey
1	0.41	0.07	10	A
2	0.36	0.06	10	A, B
3	0.33	0.07	10	B
4	0.26	0.03	10	C
5	0.31	0.05	10	B, C

Means with the same letter are not significantly different

Iron

The results of foliar testing for iron are found in Table 12. The foliar iron data for 2004 were log transformed. This was based on an improvement in the graphs of the residuals and an increase in the value of the Shapiro-Wilk test statistic from 0.8231 for untransformed data to 0.9648 for log transformed data. The results of an ANOVA with Tukey adjustment on the log transformed data are presented in Table 12. The results indicate that for 2004, foliar iron levels decrease with increasing age. In particular, trees aged 1 and 2 showed significant higher foliar iron concentrations than the trees aged 4 and 5. These results confirm previous operational observations at the ERCO tree farm that iron becomes increasingly deficient over time. This

deficiency is most likely due to a combination of soil compaction, iron ion availability, and the competition with other ions in the root zone for uptake.

Table 12. 2004 ANOVA for Foliar Iron (ppm)

Age	Mean	SD	N	Tukey
1	181.55	96.13	10	A
2	157.60	88.37	10	A, B
3	94.39	17.25	10	B, C
4	88.98	19.89	10	C
5	89.85	20.55	10	C

Means with the same letter are not significantly different

Manganese

Foliar manganese results are found in Table 13. The 2004 data for foliar manganese was log transformed based on the shape of the graph of the residuals and the improved value of 0.9908, compared to an untransformed value of 0.8900, in the value of the Shapiro-Wilk test statistic. The Levene's HOV test p-value of 0.1911 for log transformed data was greater than the p-value of 0.5 required for assumption of equal variances. The ANOVA results on the log transformed data using a Tukey adjustment are presented in Table 13. The general trend for the 2004 foliar manganese data is a decrease in foliar concentrations with age. The results indicate that the trees of ages 1 and 2 have significantly higher foliar manganese compared to the age 5 trees. Manganese availability is negatively affected by high pH. It is plausible that the high pH found in lime stabilized biosolids impacts the effectiveness of manganese uptake, especially in the older trees whose root systems fully encompass the biosolids.

Table 13. 2004 ANOVA for Foliar Manganese (ppm)

Age	Mean	SD	N	Tukey
1	177.74	127.75	10	A
2	228.09	189.77	10	A
3	159.97	177.89	10	A, B
4	73.77	41.57	10	A, B
5	52.30	26.69	10	B

Means with the same letter are not significantly different

Zinc

Foliar zinc results are found in Table 14. The foliar zinc data for 2004 were log transformed based on the distribution in the graph of the residuals. While Levene's HOV test indicates that there may be a problem with assuming equal variances, the graphs of the residuals indicate that the log transforming the data addresses the normality problem. Additionally, the value of 0.9807 for the Shapiro-Wilk statistic of log transformed data is quite satisfactory. Thus, the log transformed data was used to generate an ANOVA with a Tukey adjustment. The general trend for the 2004 foliar zinc data is a sharp decrease from age 1 to age 2, followed by a plateau from ages 2 to 5. The results, found in Table 14, indicate that while the age 3 trees have lower values for foliar zinc than all other ages of trees, only the difference between trees aged 1 and 3 is significant.

Table 14. 2004 ANOVA for Foliar Zinc (ppm)

Age	Mean	SD	N	Tukey
1	101.98	59.08	10	A
2	68.07	29.66	10	A, B
3	54.39	17.39	10	B
4	69.00	19.68	10	A, B
5	73.37	10.15	10	A, B

Means with the same letter are not significantly different

Leaf Water Potential

The leaf water potential data for 2004 are found in Table 15. Transforming the data did not result in any improvement of the test metrics. Thus, the

untransformed data were used to generate an ANOVA with a Tukey adjustment. The results of the ANOVA, found in Table 15, show that there are no significant differences between the different ages of trees for the year 2004. Compared to walnut, almond, dried plum, juniper, digger pine and incense cedar, the leaf water potential readings for hybrid poplar indicate high stress levels. High stress levels are detrimental to plant growth, resulting in stomata closure, the cessation of shoot growth, wilting and defloration. Additionally, decreased root conductivity in nutrient poor soils results in a decrease in the effectiveness of water uptake (Sands and Mulligan, 1990).

Table 15. 2004 ANOVA for Leaf Water Potential (bar)

Age	Mean	SD	N	Tukey
1	25.85	7.16	10	A
2	20.75	4.17	10	A
3	25.05	4.48	10	A
4	25.75	4.03	10	A
5	21.00	3.78	10	A

Means with the same letter are not significantly different

2005

Trees of ages 2 through 5 were sampled in 2005. Age 1 trees were not sampled due to 100% deer browse on surviving trees. The age 6 trees were not sampled due to a combination of inclement weather conditions, scheduling difficulties, and equipment failure. The results for foliar phosphorus, potassium, sulfur, iron, copper, manganese, zinc, sodium and aluminum were unremarkable and are located in Appendix 1.

Carbon

The 2005 foliar testing results for carbon are found in Table 16. Both the untransformed data and the log transformed data had a Levene's HOV test statistic p-

value greater than 0.05. None of the transformations resulted in improvements of the graphs of the residuals or in the value of the Shapiro-Wilk test statistic. Thus, the untransformed data were used in the ANOVA. The ANOVA results using a Tukey adjustment are presented in Table 16. While these results seem to indicate a significant difference between ages 4 and 5, the small sample size and the fact that the Shapiro-Wilk test statistic value of 0.8980 is less than 0.95, indicative of a non-normal distribution of treatment means which typical data transformations were unable to correct, preclude declaring the differences as significant.

Table 16. 2005 ANOVA for Foliar Carbon (%)

Age	Mean	SD	N	Tukey
2	47.22	0.41	4	A,B
3	47.18	0.19	4	A,B
4	46.73	0.20	4	A
5	47.44	0.31	4	B

Means with the same letter are not significantly different

Nitrogen

Nitrogen foliar testing results are found in Table 17. The foliar nitrogen data for 2005 was not transformed based on the evaluation of the metrics for foliar nitrogen. The results of an ANOVA with Tukey adjustment on untransformed data are found in Table 17. The general trend for 2005 appears to be a peak in foliar nitrogen at age 3 followed by a decrease through the oldest trees tested (age 5). The results indicate significantly lower foliar nitrogen levels in the age 5 trees when compared to all other ages. Similar to the 2004 data, all ages of trees in 2005 showed foliar nitrogen levels less than the 3.5% benchmark for optimal growth. Additionally, the age 5 trees show a mean value for foliar nitrogen less than 3%. This is indicative of less than adequate nutrient availability required for fast growth (Hansen, 1994). This decrease in foliar nitrogen levels of older trees matches previous observations at

the ERCO tree farm and is a good indication that the trees have utilized most of the nitrogen available in the biosolids.

Table 17. 2005 ANOVA for Foliar Nitrogen (%)

Age	Mean	SD	N	Tukey
2	3.23	0.13	4	A
3	3.30	0.11	4	A
4	3.17	0.18	4	A
5	2.87	0.12	4	B

Means with the same letter are not significantly different

Calcium

Foliar calcium results are found in Table 18. The foliar calcium data for 2005 was log transformed based on the improvement in the amount of kurtosis. However, the transformed and untransformed data showed that there were unequal variances, evident in the Levene's HOV test p-value of less than 0.0001. The results of an ANOVA with Tukey adjustment on the log transformed data are found in Table 18. The results indicate that for the year 2005, the general trend was an increase in foliar calcium levels that peaked at age 4. The age 4 trees showed significantly higher concentrations of foliar calcium levels than in all other ages.

Table 18. 2005 ANOVA for Foliar Calcium (%)

Age	Mean	SD	N	Tukey
2	1.00	0.05	4	A
3	1.15	0.06	4	A, B
4	1.74	0.13	4	C
5	1.35	0.25	4	B

Means with the same letter are not significantly different

Magnesium

The results for foliar magnesium are found in Table 19. Transformations did not improve any of the test metrics for the 2005 foliar magnesium data. While log transformation slightly improved the value of the amount of skew in the boxplot, this improvement was marginal and did not noticeably impact the graphs of the residuals.

Thus, the untransformed data were used to generate an ANOVA with a Tukey adjustment. The results are presented in Table 19 and show that the age 5 trees have significantly lower levels of foliar magnesium than the age 2 and age 3 trees. One plausible explanation for the decrease in foliar magnesium with increasing tree age is that the trees have utilized most of the magnesium available in the biosolids.

Table 19. 2005 ANOVA for Foliar Magnesium (%)

Age	Mean	SD	N	Tukey
2	0.38	0.04	4	A
3	0.35	0.03	4	A
4	0.33	0.04	4	A, B
5	0.27	0.03	4	B

Means with the same letter are not significantly different

Boron

Foliar boron results for 2005 are found in Table 20. For the 2005 data, the Levene's HOV test was greater than 0.05 allowing for the assumption of equal variances within the different ages of trees. However, log transforming the data greatly improved the Shapiro-Wilk test statistic from 0.9162 for untransformed data to 0.9615 for log transformed data without adversely affecting the value of the Levene's HOV test statistic or impacting the graphs of the distribution of the residuals. Thus, the log transformed data were used in the ANOVA. The ANOVA results using a Tukey adjustment are presented in Table 20. The general trend for the 2005 foliar boron data is an increase in concentrations with age. These results indicate that foliar boron concentrations start to become significantly different between older and younger trees starting at ages 3 or 4. The younger trees aged 2 and 3 have significantly lower foliar boron levels than older trees aged 4 and 5. This accumulation of boron with age is expected since boron a key component required for cell growth and division. Also, boron plays an important part in plant reproduction.

It is likely that as trees age, they begin to accumulate the boron that is necessary for reproductive processes.

Table 20. 2005 ANOVA for Foliar Boron (ppm)

Age	Mean	SD	N	Tukey
2	34.50	8.58	4	A
3	30.00	6.27	4	A
4	50.25	6.70	4	B
5	62.25	5.12	4	B

Means with the same letter are not significantly different

2006

Trees of ages 0 through 7 were sampled in 2006. The results for foliar zinc were unremarkable and are located in Appendix 1.

Nitrogen

The results for the 2006 foliar nitrogen are found in Table 21. An evaluation of the 2006 foliar nitrogen data showed that data transformations were not necessary. The results of an ANOVA with Tukey adjustment on the untransformed data are presented in Table 21. The trend for foliar nitrogen data for 2006 was a decrease from year 0 to year 1, followed by an increase with a peak at year 6. Ages 5 and 6 were the only trees that had foliar nitrogen levels higher than the 3.5% benchmark for optimal growth. The results indicate that the age 1 trees had significantly lower levels of foliar nitrogen than all other ages. Additionally, the age 0 and age 2 trees had significantly lower nitrogen levels than the age 6 trees. The low foliar nitrogen in year 1 trees is a result of the depletion of reserves between years 0 and 1 combined with the lack of root structure. The increase in age 2 is indicative of root structure growth that is now able to access the nutrient sources found in the biosolids. The decrease in foliar nitrogen from age 6 to age 7 is most likely due to the fact that the trees have utilized most, if not all, of the nutrients available to the trees from the

biosolids. This is the typical pattern observed at the ERCO tree farm with the exception that in previous years the peak in foliar nitrogen levels is typically seen in age 4 trees. The delay in the peak foliar nitrogen levels may be a result of slower tree growth resulting from a lack of adequate timely precipitation.

Table 21. 2006 ANOVA for Foliar Nitrogen (%)

Age	Mean	SD	N	Tukey
0	3.14	0.33	5	A
1	2.13	0.24	5	B
2	3.29	0.54	5	A
3	3.40	0.39	5	A, C
4	3.43	0.17	5	A, C
5	3.54	0.41	5	A, C
6	4.03	0.21	5	C
7	3.33	0.38	5	A, C

Means with the same letter are not significantly different

Phosphorus

Foliar phosphorus results for the 2006 data are found in Table 22. While the square root transformation of the data showed a decrease in the amounts of skew and kurtosis and an improvement in the value of the Shapiro-Wilk test, the graphs of the residuals indicated that no data transformation was necessary. Thus, the untransformed data were used in the generation of an ANOVA. The results of an ANOVA with Tukey adjustment on the untransformed data are presented in Table 22. The general trend for 2006 appears to be an initial peak at age 0 followed by a second peak at ages 5-6. These results indicate that for 2006 ages 1, 3, and 4 trees had significantly lower foliar phosphorus levels than the trees aged 5 and 6. Additionally, the age 7 trees had significantly lower foliar phosphorus levels than the age 6 trees. The initial peak found in the age zero trees is most likely due to the stecking utilizing its stores of phosphorus while becoming established. The development of the tree root system over time is the most likely explanation for the differences in foliar

phosphorus levels with respect to age. The more developed root systems found in older trees are better suited at utilizing different nutrient sources for phosphorus uptake. Mean ideal foliar phosphorus levels in excess of the 0.30% benchmark for rapid growth were observed in ages 0, 2, 5, 6 and 7 trees.

Table 22. 2006 ANOVA for Foliar Phosphorus (%)

Age	Mean	SD	N	Tukey
0	0.36	0.06	5	A, B, C
1	0.28	0.03	5	B
2	0.36	0.01	5	A, B, C
3	0.29	0.07	5	B
4	0.28	0.05	5	B
5	0.42	0.08	5	A, C
6	0.47	0.05	5	A
7	0.35	0.05	5	B, C

Means with the same letter are not significantly different

Potassium

Foliar potassium results for 2006 are found in Table 23. Compared to untransformed data, log transformation of the data resulted in some improvements in the values of the Shapiro-Wilk test statistic, kurtosis and skew. However, the p-value for the Levene's HOV test decreased from 0.0610 for untransformed data to 0.0238 for log transformed data. Since log transformation significantly decreased the value for the Levene's HOV test and did not significantly alter the values for the other performance metrics, the untransformed data were used to generate an ANOVA. The results of the ANOVA on untransformed data with Tukey adjustment, found in Table 23, show that for 2006 there were a number of significant differences between the different ages of trees. However, the range in values for the different ages makes interpreting the differences between them difficult. It appears that for the 2006 foliar potassium data, the general trend is an increase in foliar potassium levels as the trees age, with concentrations peaking in age 6. One possible explanation for the wide

range of values for the different ages and the generally higher values overall when compared to the other years in the study is rainfall. Over 2 inches of rain fell at the ERCO tree farm in the two weeks prior to foliar testing. Potassium is very mobile in plants, and potassium uptake rates may have increased during this period due to the increased availability of potassium in the soil as a result of the influx of water. The higher foliar potassium concentrations observed in the older trees are most likely a result of the larger and better developed root systems found in older trees.

Table 23. 2006 ANOVA for Foliar Potassium (%)

Age	Mean	SD	N	Tukey
0	3.33	0.32	5	A, B
1	1.85	0.26	5	C
2	2.77	0.17	5	B, D
3	1.88	0.24	5	C
4	2.45	0.88	5	C, D
5	2.63	0.26	5	B, C, D
6	3.77	0.29	5	A
7	3.04	0.52	5	A, B, D

Means with the same letter are not significantly different

Calcium

Foliar calcium results for 2006 are found in Table 24. Log transformation of the foliar data showed a decrease in the amounts of skew and kurtosis and an increase in the value of the Shapiro-Wilk test compared to untransformed data. The results of an ANOVA with Tukey adjustment on the log transformed data are presented in Table 24. The general trend for the 2006 appears to be a stable foliar calcium level at or around 1.0%. The results indicate that for 2006 foliar calcium levels were significantly lower in age 3 trees than in ages 0, 1, and 4 trees. Additionally, age 4 trees had significantly higher foliar calcium levels than age 5 trees.

Table 24. 2006 ANOVA for Foliar Calcium (%)

Age	Mean	SD	N	Tukey
0	1.07	0.05	5	A, B
1	1.07	0.15	5	A, B
2	1.02	0.13	5	A, B, C
3	0.80	0.17	5	C
4	1.22	0.23	5	A
5	0.89	0.12	5	B, C
6	1.06	0.13	5	A, B, C
7	0.92	0.12	5	A, B, C

Means with the same letter are not significantly different

Magnesium

Foliar magnesium results are found in Table 25. Data transformations did not result in any improvements of the performance metrics for the 2006 foliar magnesium data. Untransformed data were used in an ANOVA with a Tukey adjustment. The results in Table 25 indicate that for the year 2006 foliar magnesium levels peaked in trees ages 3 and 4. The trees aged 3 and 4 had significantly higher magnesium concentrations than all other ages of trees with the exception of the age 6 trees.

Table 25. 2006 ANOVA for Foliar Magnesium (%)

Age	Mean	SD	N	Tukey
0	0.20	0.05	5	A
1	0.24	0.04	5	A, B
2	0.21	0.03	5	A, B
3	0.38	0.06	5	C
4	0.37	0.07	5	C
5	0.27	0.02	5	A, B
6	0.30	0.03	5	B, C
7	0.26	0.03	5	A, B

Means with the same letter are not significantly different

Sulfur

Foliar sulfur results for 2006 are found in Table 26. Transformation was not required for the 2006 foliar sulfur data based an evaluation of the performance metrics. However, the p-value for the Levene's HOV test for both the transformed and untransformed foliar sulfur data indicated that there were unequal variances.

Additionally, the p-value for the Welch's test for transformed and untransformed data indicated that there were unequal means. Keeping these limitations in mind, an ANOVA with Tukey adjustment was performed on the untransformed data. The results, presented in Table 26, indicate that the foliar sulfur levels for age 1 trees are significantly lower than all other ages of trees except for the age 3 trees. The lower foliar sulfur levels in the age 1 trees is most likely a result of the lack of root system development in the younger trees.

Table 26. 2006 ANOVA for Foliar Sulfur (%)

Age	Mean	SD	N	Tukey
0	0.39	0.03	5	A
1	0.24	0.01	5	B
2	0.33	0.06	5	A
3	0.32	0.07	5	A, B
4	0.35	0.02	5	A
5	0.34	0.04	5	A
6	0.41	0.01	5	A
7	0.33	0.04	5	A

Means with the same letter are not significantly different

Iron

The foliar iron results for 2006 are found in Table 27. While the boxplot graph of the untransformed data indicates some skew, data transformations only resulted in marginal improvements of the performance metrics. Thus, the untransformed data were used in the ANOVA. The results of the ANOVA with Tukey adjustment on the untransformed data are presented in Table 27. While the general trend appears to be a peak in foliar iron levels at age 3 followed by a decline in foliar iron levels with increasing tree age, the only significant difference between the different ages of trees for the 2006 foliar iron data was that the age 3 trees have higher foliar iron levels than the age 6 trees. Similar to the 2004 results, the results of 2007 agree with previous operational observations at the ERCO tree farm that iron

becomes increasingly deficient over time. One notable difference between 2004 and 2007 is that the foliar iron levels start off at much lower levels in the younger trees in 2007. While a combination of soil compaction, iron ion availability, and the competition with other ions in the root zone for uptake are the most likely explanations for the iron deficiency levels observed, it is possible that the differing amounts of rainfall played an important role in exacerbating iron deficiency.

Table 27. 2006 ANOVA for Foliar Iron (ppm)

Age	Mean	SD	N	Tukey
0	90.00	12.00	5	A, B
1	109.40	29.80	5	A, B
2	81.80	8.41	5	A, B
3	125.40	31.03	5	A
4	89.60	18.69	5	A, B
5	85.00	6.75	5	A, B
6	76.00	27.31	5	B
7	95.00	26.90	5	A, B

Means with the same letter are not significantly different

Copper

The results for the 2006 foliar copper data are found in Table 28. The graphs of the residuals for the 2006 data did not indicate that a transformation was necessary. Additionally, the log and square root data transformations did not improve the value of the Shapiro-Wilk test statistic or the p-value for Levene's HOV test. Thus, the untransformed data were used in the ANOVA. The results of an ANOVA with Tukey adjustment on the untransformed data are presented in Table 28. The general trend of the data is a plateau in foliar copper values around 10.50 ppm after age 1. The data indicates that age 1 trees have significantly lower foliar copper levels than the trees of ages 0, 2, 4, 6, and 7. Additionally, the trees of age 1 have lower, but not significantly lower, foliar copper levels than trees ages 3 and 5. A possible explanation for the lower foliar copper levels observed in the age 1 trees is that the

trees may have depleted any storage reserves between ages 0 and 1 as they work to establish their root systems. It appears that copper uptake is not an ongoing problem once the root system of the tree becomes established.

Table 28. 2006 ANOVA for Foliar Copper (ppm)

Age	Mean	SD	N	Tukey
0	11.20	0.84	5	A
1	7.40	2.07	5	B
2	10.20	1.30	5	A
3	9.40	0.89	5	A, B
4	11.40	1.67	5	A
5	8.80	0.84	5	A, B
6	10.40	0.55	5	A
7	10.40	1.52	5	A

Means with the same letter are not significantly different

Manganese

Foliar manganese results for the year 2006 are found in Table 29. Log transforming of the data was utilized based on the improvement in the value of the Shapiro-Wilk test statistic to 0.9557 for log transformed data from 0.9294 for untransformed data. The ANOVA results using a Tukey adjustment on the log transformed data are presented in Table 29. While the foliar manganese results for 2006 are somewhat ambiguous, the data appears to indicate a general trend of younger (ages 0, 1, and 2) and older (ages 5 and 7) trees having similar levels of foliar manganese.

Table 29. 2006 ANOVA for Foliar Manganese (ppm)

Age	Mean	SD	N	Tukey
0	58.60	24.72	5	A
1	84.20	34.69	5	A, B
2	63.60	46.54	5	A
3	129.60	30.97	5	B
4	147.80	58.40	5	B
5	76.60	22.48	5	A, B
6	133.60	20.57	5	B
7	74.80	36.07	5	A, B

Means with the same letter are not significantly different

Boron

Foliar boron results for 2006 are found in Table 30. The samples were log transformed, resulting in a Levene's HOV test statistic p-value of 0.4377 and a Shapiro-Wilk test statistic value of 0.9802. The results of an ANOVA using a Tukey adjustment on the log transformed data are presented in Table 30. The results of the ANOVA indicate that the younger trees are different than the older trees starting at ages 3 and 4. The data also seem to indicate that foliar boron levels peak and possibly plateau at age 5. There are two possible explanations for these observations. The first possible explanation is that the boron uptake and usage may be shifting from growth pathways into reproduction mechanisms. Though hybrid poplars are sterile and they are generally harvested before reaching reproductive age, the mechanisms are still in place for increased boron requirements for seed production and other reproductive needs. The second explanation revolves around the ability of the trees to uptake boron from the soil. Slower growth as a result of drought conditions affects the rate of root growth, which in turn may result in lower levels of boron uptake. The end result is that lower amounts of boron are available to the tree for uptake, and this in turn is expressed as a decrease in foliar boron levels.

Table 30. 2006 ANOVA for Foliar Boron (ppm)

Age	Mean	SD	N	Tukey
0	26.60	2.07	5	A, B
1	28.40	3.36	5	A,B
2	31.40	4.83	5	A,B
3	24.20	5.50	5	A
4	32.80	5.07	5	B, C
5	46.20	6.46	5	D
6	42.60	4.72	5	C,D
7	35.40	3.65	5	B,C,D

Means with the same letter are not significantly different

Sodium

The results for foliar sodium in 2006 are found in Table 31. The values for Levene's HOV test and the Shapiro-Wilk test were identical for untransformed, log transformed, and square root transformed data. The Levene's HOV test p-value of 0.1157 of the data set was greater than 0.05 and this is indicative of equal variances within each age. However, the Shapiro-Wilk test statistic value of 0.9040 for the data set was less than 0.95. This is indicative of non-normal distribution of treatment means. These values are most likely affected by the very low levels of variation between samples and the small sample size. Bearing these limitations in mind, the untransformed data were used in the ANOVA, and the results of the ANOVA using a Tukey adjustment are presented in Table 31. While there are some significant differences between ages, there does not appear to be a discernable overall trend with respect to foliar sodium levels. It is possible that the variations between the different ages are a result of localized environmental influences, in particular the amount of water available.

Table 31. 2006 ANOVA for Foliar Sodium (%)

Age	Mean	SD	N	Tukey
0	0.018	0.004	5	A
1	0.012	0.004	5	A, B
2	0.014	0.005	5	A, B
3	0.010	0.000	5	B
4	0.014	0.005	5	A, B
5	0.010	0.000	5	B
6	0.010	0.000	5	B
7	0.012	0.004	5	A, B

Means with the same letter are not significantly different

Aluminum

Foliar aluminum results for 2006 are found in Table 32. The graphs of the residuals did not clearly suggest transforming the data. Untransformed data were

used due to the p-value of Levene's HOV test (0.0767) compared to the p-values for the different transformations. The results of an ANOVA with a Tukey adjustment on the untransformed data are presented in Table 32. The results indicate that age 0 trees have significantly higher foliar aluminum levels than trees of ages 2, 3, 5, 6, and 7. The general trend evident for the year 2006 is a decrease in foliar aluminum with increasing tree age.

Table 32. 2006 ANOVA for Foliar Aluminum (ppm)

Age	Mean	SD	N	Tukey
0	26.80	4.87	5	A
1	20.40	4.77	5	A, B
2	11.80	2.49	5	B, C
3	13.80	2.39	5	B, C
4	16.20	8.20	5	A, B, C
5	9.20	5.59	5	B, C
6	7.00	1.41	5	C
7	14.40	10.38	5	B, C

Means with the same letter are not significantly different

2007

Trees of ages 2 and ages 4 through 7 were sampled in 2007. Deer browse, heat and drought resulted in levels of mortality that precluded sampling trees of ages 0, 1, and 3. The results for foliar potassium, copper, zinc and sodium were unremarkable and are located in Appendix 1.

Nitrogen

The 2007 foliar nitrogen results are found in Table 33. Transformations on the data resulted in no or marginal improvements on the performance metrics. Thus, the untransformed data were used in the ANOVA. The results of an ANOVA with Tukey adjustment on the untransformed data is found in Table 33. The results indicate that for 2007 the age 7 trees had a significantly lower foliar nitrogen level than the trees aged 4 and 5. The trend for the 2007 foliar nitrogen data indicates a

peak at age 4 followed by decreasing foliar nitrogen concentrations with age until age 7. Foliar nitrogen peaking at or around age 4 is the typical pattern of foliar nitrogen levels observed previously during typical operations at the ERCO tree farm. This is a result of the trees utilizing all of the easily accessible nitrogen from the biosolids. All ages of trees had foliar nitrogen levels lower than the 3.5% benchmark for rapid growth. Additionally, the trees of age 7 show a foliar nitrogen level much lower than 2.7% which is indicative of nitrogen deficiency.

Table 33. 2007 ANOVA for Foliar Nitrogen (%)

Age	Mean	SD	N	Tukey
2	2.86	0.26	5	A, B
4	3.25	0.17	5	A
5	3.13	0.24	5	A
6	2.90	0.19	5	A, B
7	2.51	0.29	4	B

Means with the same letter are not significantly different

Phosphorus

The results for the 2007 foliar phosphorus data are found in Table 34. Similar to the data from 2006, square root transformation of the 2007 foliar phosphorus data showed a decrease in the amount of skew and kurtosis and a very slight decrease in the value of the Shapiro-Wilk test. However, the graphs of the residuals indicated that no data transformations were necessary. Thus, untransformed data were used in the generation of an ANOVA. The results of an ANOVA with Tukey adjustment on the untransformed data are presented in Table 34. These results indicate that for 2007 the age 2 trees had significantly higher foliar phosphorus concentrations than trees ages 4, 5, and 7. Additionally, the age 4 trees had significantly lower foliar phosphorus levels than the age 6 trees. All ages had mean foliar phosphorus levels much lower than the 0.30% ideal benchmark for rapid growth. One possible

explanation for the lower phosphorus values overall is the impact that the lack of rainfall would have on soil pH and ion competition. Phosphorus availability in the soil is highest under conditions where soil moisture is at or near field capacity (Landis and van Steenis, 2004b). The months of June and July saw a combined total of 3.61 inches of rainfall at the ERCO site. It is possible that the lack of rainfall coupled with the elevated pH in and around the biosolids resulted in other ions, in particular calcium, iron, and aluminum, forming non-labile compounds with phosphorus. This would decrease the amount of phosphorus that is available to the tree for uptake and result in lower foliar phosphorus values.

Table 34. 2007 ANOVA for Foliar Phosphorus (%)

Age	Mean	SD	N	Tukey
2	0.18	0.03	5	A
4	0.14	0.02	5	B
5	0.15	0.01	5	B, C
6	0.17	0.02	5	A, C
7	0.15	0.01	4	B, C

Means with the same letter are not significantly different

Calcium

Foliar calcium results are found in Table 35. Transformations on the 2007 data resulted in marginal improvements on the performance metrics. Thus, the untransformed data were used in an ANOVA with Tukey adjustment, and these results are found in Table 35. In 2007, age 4 trees had significantly lower foliar calcium levels than all other ages of trees. Additionally, age 7 trees had significantly higher foliar calcium levels than trees ages 2, 4, and 5.

Table 35. 2007 ANOVA for Foliar Calcium (%)

Age	Mean	SD	N	Tukey
2	1.93	0.20	5	A
4	1.08	0.19	5	B
5	1.72	0.37	5	A
6	2.16	0.31	5	A, C
7	2.47	0.12	4	C

Means with the same letter are not significantly different

Magnesium

Foliar magnesium results are found in Table 36. Similar to 2005 and 2006, transforming the data did not improve any of the performance. Thus, the untransformed data were used to generate an ANOVA with a Tukey adjustment. These results are found in Table 36. The 2007 data indicates that the age 4 trees have significantly higher foliar magnesium levels than the trees of ages 2 and 7. While there are no significant differences between trees ages 4, 5, and 6, the general trend for the 2007 data appears to be that foliar magnesium levels peak at age 4 and decrease with increasing tree age.

Table 36. 2007 ANOVA for Foliar Magnesium (%)

Age	Mean	SD	N	Tukey
2	0.28	0.07	5	A
4	0.50	0.10	5	B
5	0.40	0.03	5	A, B, C
6	0.42	0.06	5	B, C
7	0.31	0.02	4	A, C

Means with the same letter are not significantly different

Sulfur

Foliar sulfur results for 2007 are found in Table 37. Similar to the previous years, data transformations were not required. Thus, untransformed data was used to generate an ANOVA with Tukey adjustment. These results, presented in Table 37, indicate that the 2007 foliar sulfur levels were significantly lower in the age 4 trees than in the trees aged 2, 6, and 7. Compared to previous years, the lower levels of

foliar sulfur observed in the age 4 trees are most likely a sampling anomaly. Additionally, the age 7 trees have significantly higher foliar sulfur levels than all other ages of trees except for age 6 trees. This is most likely a result of a fully developed root system that is able to incorporate sulfur much easier under less than ideal environmental conditions.

Table 37. 2007 ANOVA for Foliar Sulfur (%)

Age	Mean	SD	N	Tukey
2	0.38	0.02	5	A
4	0.27	0.03	5	B
5	0.34	0.05	5	A, B
6	0.39	0.05	5	A, C
7	0.46	0.04	4	C

Means with the same letter are not significantly different

Iron

Foliar iron results for 2007 are found in Table 38. An analysis of the performance metrics on the transformed data indicated that the transformations used did not result in any improvement when compared to untransformed data. Thus, an ANOVA with Tukey adjustment was performed on the untransformed data, and these results are presented in Table 38. The only significant difference in foliar iron levels for 2007 was between trees ages 2 and 6. Similar to 2006, the general trend for foliar iron levels in 2007 appears to be a peak in younger trees followed by a decline in foliar iron concentrations in older trees. However, the peak for the 2007 year is in the age 2 trees whereas the peak for 2006 was in age 3 trees. This may be a result of the fact that there were no living age 3 trees to sample in 2007 due to a combination of deer browse and drought.

Table 38. 2007 ANOVA for Foliar Iron (ppm)

Age	Mean	SD	N	Tukey
2	111.60	9.91	5	A
4	96.80	17.78	5	A, B
5	94.60	11.08	5	A, B
6	78.80	12.46	5	B
7	101.50	17.71	4	A, B

Manganese

The foliar manganese results for 2007 are found in Table 39. Log transformation was used on the raw 2007 data. This was based on the improvement of the Levene's HOV test p-value from 0.0054 for untransformed to 0.1797 for log transformed data. The results of the ANOVA with a Tukey adjustment on log transformed data are presented in Table 39. The results indicate that for 2007 the age 2 trees have significantly lower foliar manganese levels than trees ages 4, 5, 6, and 7. Additionally, there also appears to be a peak in foliar manganese levels at age 5, with trees of age 5 having significantly higher foliar manganese levels than ages 2 and 7. However, this peak may be a result of the large standard deviation for the age 5 data compared to the other ages.

Table 39. 2007 ANOVA for Foliar Manganese (ppm)

Age	Mean	SD	N	Tukey
2	103.80	51.45	5	A
4	231.80	18.86	5	B, C
5	378.80	132.77	5	B
6	228.00	36.65	5	B, C
7	180.40	23.30	4	C

Means with the same letter are not significantly different

Boron

Foliar boron results are found in Table 40. The samples tested in 2007 were log transformed, resulting in a Levene's HOV test statistic p-value of 0.2224 and a Shapiro-Wilk test statistic value of 0.9565. The results of an ANOVA using a Tukey

adjustment are presented in Table 40. The ANOVA indicated that the oldest trees, ages 6 and 7, have significantly higher foliar boron levels than all other trees. This is probably a result of older trees having a more expansive root network that allows for increased levels of boron uptake. Unlike previous years, there is not a peak in foliar boron levels at age 5. It is possible that the differences in foliar boron uptake from year to year are a result of the environmental impact of water availability, either locally or on a plantation scale.

Table 40. 2007 ANOVA for Foliar Boron (ppm)

Age	Mean	SD	N	Tukey
2	35.00	6.24	5	A,B
4	28.00	6.28	5	B
5	39.40	6.31	5	A
6	70.80	13.57	5	C
7	69.20	5.22	5	C

Means with the same letter are not significantly different

Aluminum

The results for foliar aluminum are found in Table 41. Log transformation of the data resulted in a higher Levene's HOV p-value (0.1028), a higher Shapiro-Wilk value (0.9621), and improved the distribution in the graphs of the residuals. The results of an ANOVA with a Tukey adjustment on log transformed data are presented in Table 41. The only significant difference for the year 2007 was that age 6 trees had significantly lower foliar aluminum levels than age 7 trees.

Table 41. 2007 ANOVA for Foliar Aluminum (ppm)

Age	Mean	SD	N	Tukey
2	26.20	3.63	5	A, B
4	26.20	1.64	5	A, B
5	23.40	4.83	5	A, B
6	18.00	4.64	5	B
7	35.40	15.79	4	A

Means with the same letter are not significantly different

2004-2007

Carbon

The foliar carbon data sets for 2004 and 2005, the only years of sampling, were pooled together. These results are graphed in Figure 7. While Figure 7 suggests that there might be a difference between the two years in addition to an overall downward trend, these differences are not significant. Transformations of the foliar carbon data set did not improve the graphs of the residuals or the value of the Shapiro-Wilk test statistic. The untransformed data had a Levene's HOV test statistic p-value of 0.6860, greater than the 0.05 value needed to assume equal variances within each age, and a Shapiro-Wilk test statistic of 0.9639, greater than 0.95 and indicative of normal distribution. Thus, untransformed data were used in the ANOVA. The ANOVA results using a Tukey adjustment are presented in Table 42. These results indicate that there is no significant difference between different ages with respect to foliar carbon concentrations for the years 2004-2005. Foliar carbon analysis was discontinued after 2005.

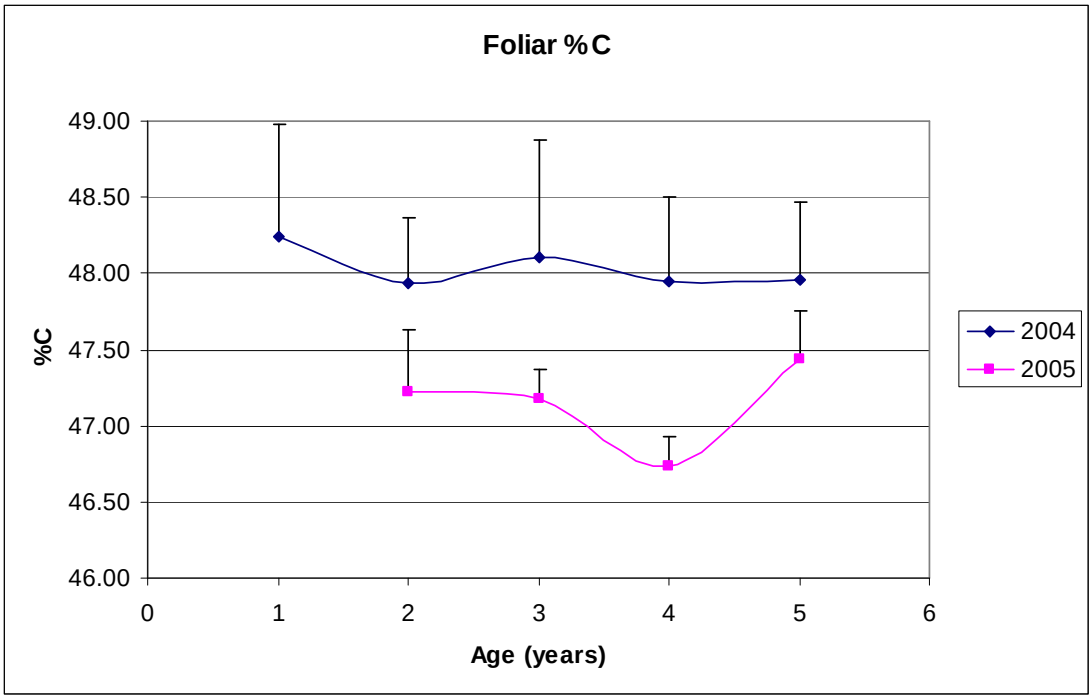

Figure 7. Foliar Carbon Concentrations for 2004-2005

Table 42. 2004-2005 ANOVA for Foliar Carbon (%)

Age	Mean	SD	N	Tukey
1	48.24	0.74	10	A
2	47.73	0.53	14	A
3	47.84	0.78	14	A
4	47.60	0.74	14	A
5	47.81	0.51	14	A

Means with the same letter are not significantly different

Nitrogen

The foliar nitrogen data for 2004-2007 were pooled together and analyzed. These results were graphed and are found in Figure 8. The transformed and untransformed data showed that there were unequal variances, evident in the Levene's HOV test p-value of less than 0.0001. Similarly, the results of the Welch's test also had a p-value of less than 0.0001, indicative of unequal variances. Bearing these limitations in mind, an ANOVA with Tukey adjustment on untransformed data was performed and the results are found in Table 43. These results indicate that over the course of the study the age 1 trees had significantly lower foliar nitrogen levels

than all other ages and that age 6 trees had significantly higher foliar nitrogen levels than age 7 trees. The lower foliar nitrogen observed in the age 1 trees is most likely attributable to a smaller root system that is just beginning to develop. As the trees grow older, their root systems grow larger and are better able to access nutrients from a wider range of sources. Additionally, the roots increasingly encompass the biosolids more and more as the trees grow older, completely surrounding the biosolids by age 4. The decline in foliar nitrogen levels beyond age 5 is most likely a result of the trees exhausting the easily available nitrogen in the biosolids. Previous mining operations at the ERCO site removed the A soil horizon, resulting in a nutrient poor soil with little or no organic matter. The biosolids provide the majority of the nutrients for the trees, and previous observations indicate that the nitrogen in the biosolids becomes depleted after about four years. The decrease in the amount of nitrogen available in the biosolids is reflected in the decreasing foliar nitrogen levels observed in the older trees. With the exception of ages 5 and 6 in 2006, all other ages of trees show mean foliar nitrogen levels below the 3.5% benchmark for optimal growth. This is indicative of less than ideal growing conditions for hybrid poplar occurring during the course of this study. It is also important to note that nitrogen levels affect other nutrients. Excess nitrogen may induce deficiencies of phosphorus, sulfur, boron, iron and copper (Brown, 1999). Similarly, induced or site specific deficiencies of these minerals may result in erratic nitrogen levels (Brown, 1999).

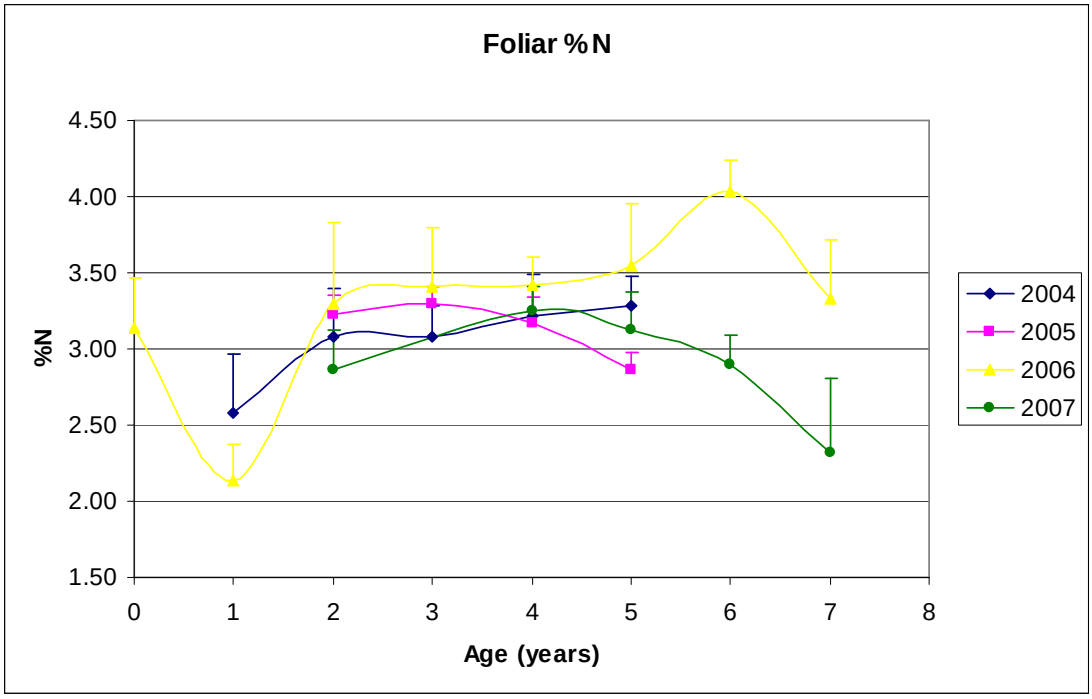

Figure 8. Foliar Nitrogen Concentrations 2004-2007

Table 43. 2004-2007 ANOVA for Foliar Nitrogen (%)

Age	Mean	SD	N	Tukey
0	3.14	0.33	5	A, B
1	2.43	0.40	15	C
2	3.10	0.36	24	A, B
3	3.21	0.28	19	A, B
4	3.26	0.23	24	A, B
5	3.24	0.32	24	A, B
6	3.47	0.63	10	A
7	2.97	0.54	9	B

Means with the same letter are not significantly different

Phosphorus

The foliar phosphorus data for 2004-2007 were pooled together and analyzed. The graphs for the mean foliar phosphorus by year are found in Figure 9. The transformed and untransformed data showed that there were unequal variances, evident in the Levene's HOV test p-value of less than 0.0001. A Welch's test also showed unequal variances. Untransformed data had the highest value for the Welch's test at 0.0328. The results of an ANOVA with Tukey adjustment on untransformed

data are found in Table 44. The results indicate that the only significant difference over the course of the study was that age 1 trees had significantly lower foliar phosphorus than age 0 trees. This difference may be a result of sampling the age 0 trees only in 2006, resulting in a small sample for only one of the four years of the study. It is also possible that this difference is a result of nutrient reserves being utilized in order for the trees to establish a root system. This may also be compounded by the different average amounts of rainfall for each year. Lack of adequate moisture would have a negative impact on both plant growth and phosphorus uptake. The general trend for foliar phosphorus over the course of the study is a sharp decrease in foliar concentrations between the ages of 0 and 1, followed by a slow increase in foliar concentrations that appears to peak at ages 5-6. Differing average rainfall amounts, and its implications, must be taken into account when interpreting the overall trend for the four years of the study. It should be noted that ages 0, 5, and 6 are the only ages that show a mean foliar phosphorus level above the 0.30% benchmark needed for fast growth. This is most likely a result of the age 0 trees utilizing stored phosphorus while becoming established. The ideal foliar phosphorus levels found in the older trees are most likely related to the development of the root system to the point where the tree is able to better access and utilize the phosphorus found in the biosolids.

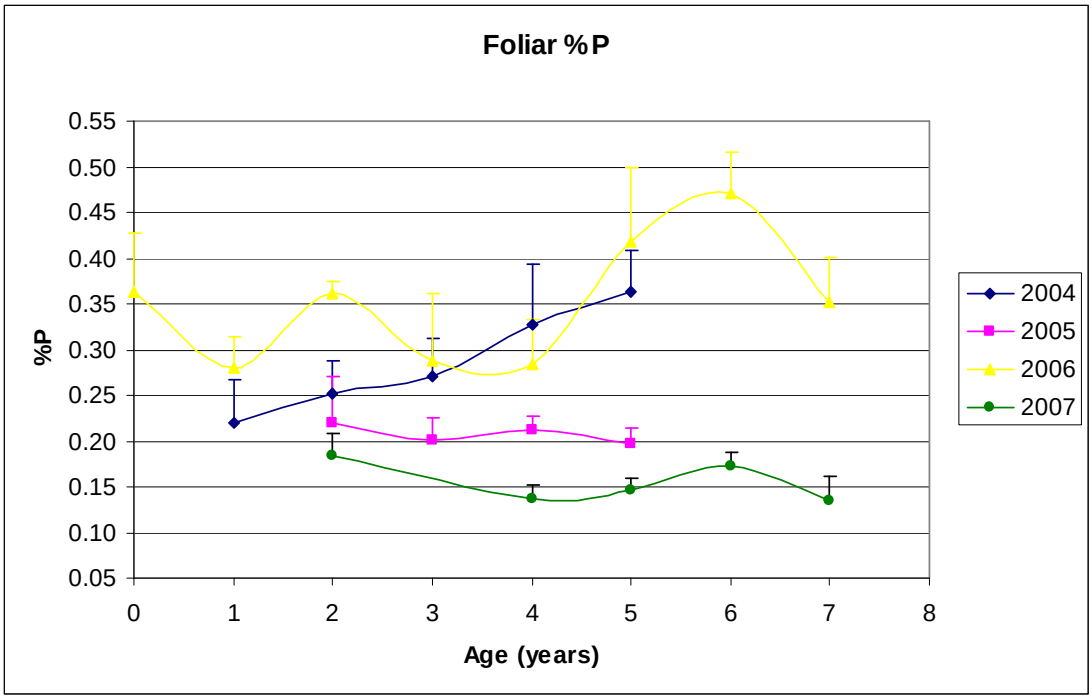

Figure 9. Foliar Phosphorus Concentrations 2004-2007

Table 44. 2004-2007 ANOVA for Foliar Phosphorus (%)

Age	Mean	SD	N	Tukey
0	0.36	0.06	5	A
1	0.24	0.05	15	B
2	0.26	0.07	24	A, B
3	0.26	0.06	19	A, B
4	0.26	0.09	24	A, B
5	0.30	0.12	24	A, B
6	0.32	0.16	10	A, B
7	0.26	0.11	9	A, B

Means with the same letter are not significantly different

Potassium

Foliar potassium data for the years 2004-2007 were pooled and evaluated. The graphs of the yearly means are found in Figure 10. Log transformation resulted in improvements for the values of the Shapiro-Wilk test statistic, kurtosis, and skew. However, Levene's HOV test for both the untransformed and transformed data showed unequal variances. The Welch's test was also indicative of heterogeneous age means. Keeping these issues in mind, an ANOVA with Tukey adjustment was

performed on log transformed data. The results, found in Table 45, show that the only significant difference between the different ages of trees over the course of the study was that age 0 trees had significantly higher levels of foliar potassium than all other ages. Ignoring age 0 trees, the overall trend for foliar potassium levels over the course of the study appears to be an increase in foliar concentrations with age, with foliar levels for the trees peaking around age 6. The higher foliar potassium levels observed in the age 0 trees are probably a result of a number of different factors. First, environmental factors, specifically rainfall amounts, most likely had an impact on foliar levels. The amounts of rainfall for 2006 are very different when compared to the years 2005 and 2007. Second, the sample size is small and limited to only one year (2006). The limited sampling makes interpretation over the course of the study questionable. Finally, it is possible that the increase in foliar potassium levels is a result of the use of steckings. It is conceivable that the higher levels of foliar potassium are a result of the stecking using previously stored potassium while it works to establish itself during the first year.

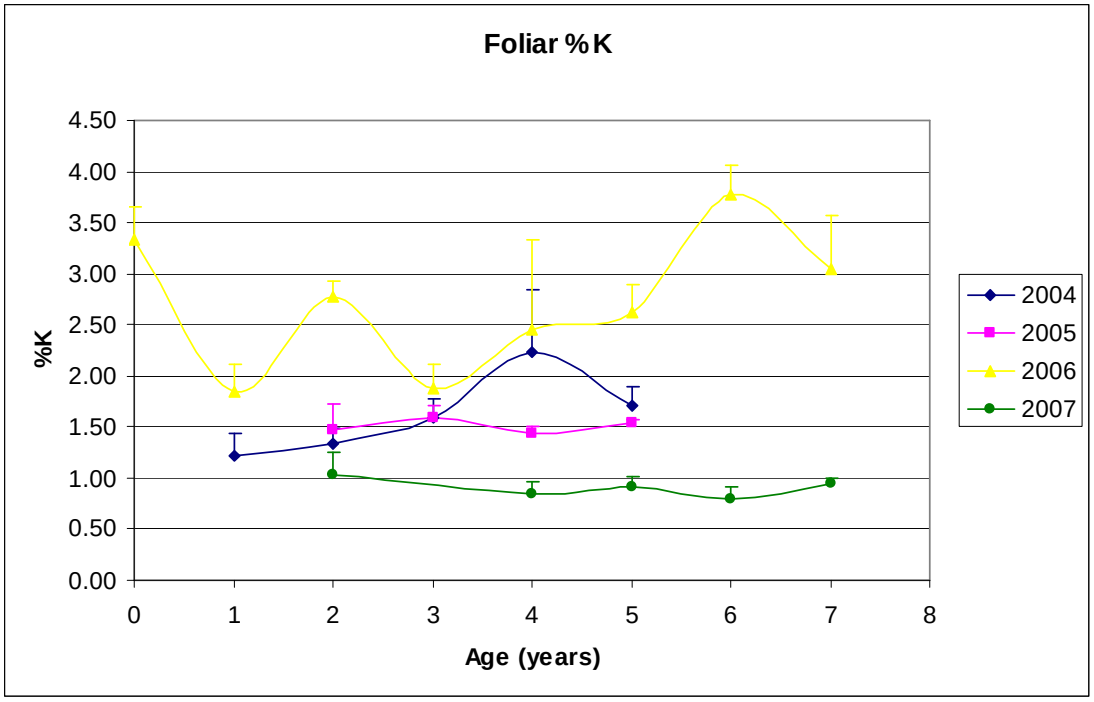

Figure 10. Foliar Potassium Concentrations 2004-2007

Table 45. 2004-2007 ANOVA for Foliar Potassium (%)

Age	Mean	SD	N	Tukey
0	3.33	0.32	5	A
1	1.43	0.38	15	B
2	1.59	0.66	24	B
3	1.66	0.23	19	B
4	1.86	0.82	24	B
5	1.70	0.60	24	B
6	2.28	1.58	10	B
7	2.12	1.16	9	B

Means with the same letter are not significantly different

Calcium

The foliar calcium data for 2004-2007 were pooled together and analyzed. Figure 11 shows the graphs of the year means. Log transforming the data improved the graphs of the residuals and decreased the amount of skew from 0.6707 to 0.2929. However, similar to the data for 2005, the transformed and untransformed data showed that there were unequal variances, evident in the Levene's HOV test p-value of less than 0.0001. The results of an ANOVA with Tukey adjustment on log

transformed data are found in Table 46. The results indicate that over the course of the study, the only significant differences between the different ages of trees appears to be that age 3 trees have significantly lower foliar calcium levels than age 6 trees. The mean values for foliar calcium observed are at the lower end of typical foliar calcium levels. It is possible that the calcium fluctuations observed at the ERCO site are due to a number of interactions. One possibility is that excess levels of other nutrients, especially nitrogen in the form of ammonium, are limiting calcium uptake. The next possibility is that soil pH is also preventing calcium uptake by increasing competition for calcium ions with other elements, such as iron and zinc. Finally, it is possible that the foliar sampling regimen, timed to capture stable levels of foliar nitrogen, does not a represent a suitable sampling time for foliar calcium. Foliar calcium levels do increase as senescence approaches, and variation between years, especially with respect to the marked increase in foliar calcium levels for older trees in 2007, may be a result of capturing the onset of senescence.

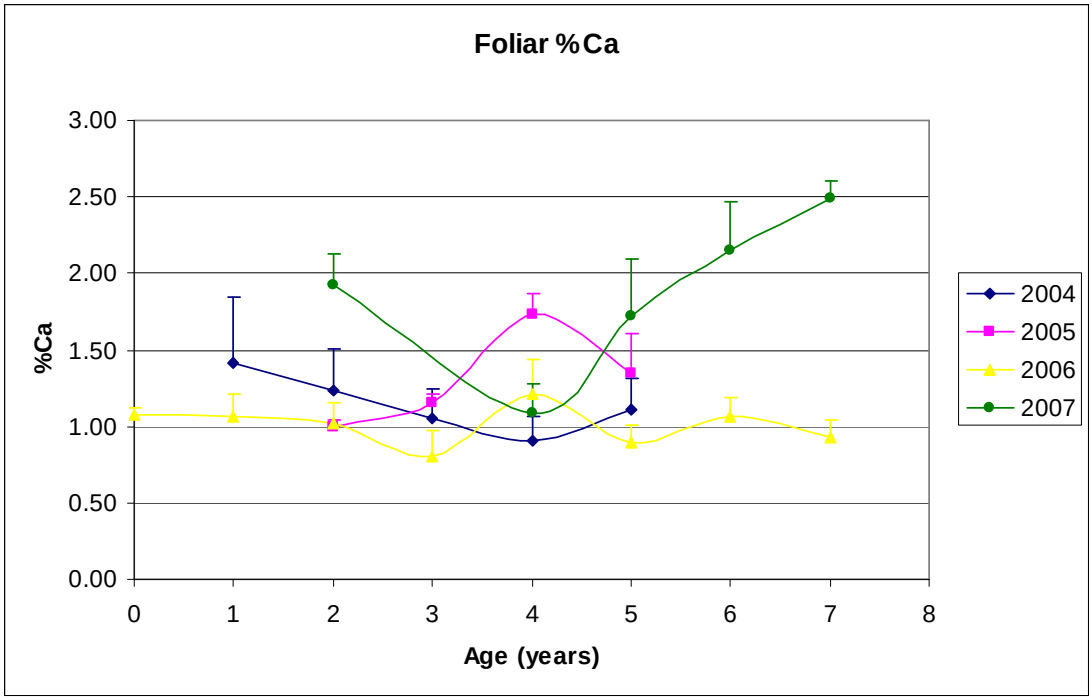

Figure 11. Foliar Calcium Concentrations 2004-2007

Table 46. 2004-2007 ANOVA for Foliar Calcium (%)

Age	Mean	SD	N	Tukey
0	1.07	0.05	5	A, B
1	1.30	0.4	15	A, B
2	1.29	0.4	24	A, B
3	1.01	0.21	19	B
4	1.15	0.34	24	A, B
5	1.23	0.37	24	A, B
6	1.61	0.62	10	A
7	1.61	0.82	9	A, B

Means with the same letter are not significantly different

Magnesium

The foliar magnesium data for 2004-2007 were pooled together and analyzed. The graphs for the year to year means are found in Figure 12. Log transforming the data improved skew, kurtosis, and the graphs of the residuals. The p-value of 0.04 for Levene's HOV test on log transformed data was less than the 0.05 needed for significance. However, the value of the Shapiro-Wilk test statistic for log transformed data was acceptable at 0.9952. Thus, log transformed data were used in

an ANOVA with Tukey adjustment. The results, presented in Table 47, indicate that the only significant difference in foliar magnesium levels is that age 0 trees have lower levels of foliar magnesium than all other ages. This observation may be due in part to the limited sample size since the age 0 trees were only sampled in 2006. The general trend for foliar magnesium appears to be a decrease in foliar magnesium levels with increasing tree age. One possible explanation for this observation is that the amount of magnesium available to the trees in the biosolids decreases over time resulting in lower foliar magnesium concentrations. It is also conceivable that environmental factors, particularly the amount of rainfall, played a role in the year to year variation in foliar magnesium levels and in the appearance of a general decreasing trend. Finally, since magnesium is mobile in plant tissue, moving from older to younger leaves, and foliar testing is performed on younger leaves, it is conceivable that foliar testing does not adequately measure plant magnesium deficiency levels.

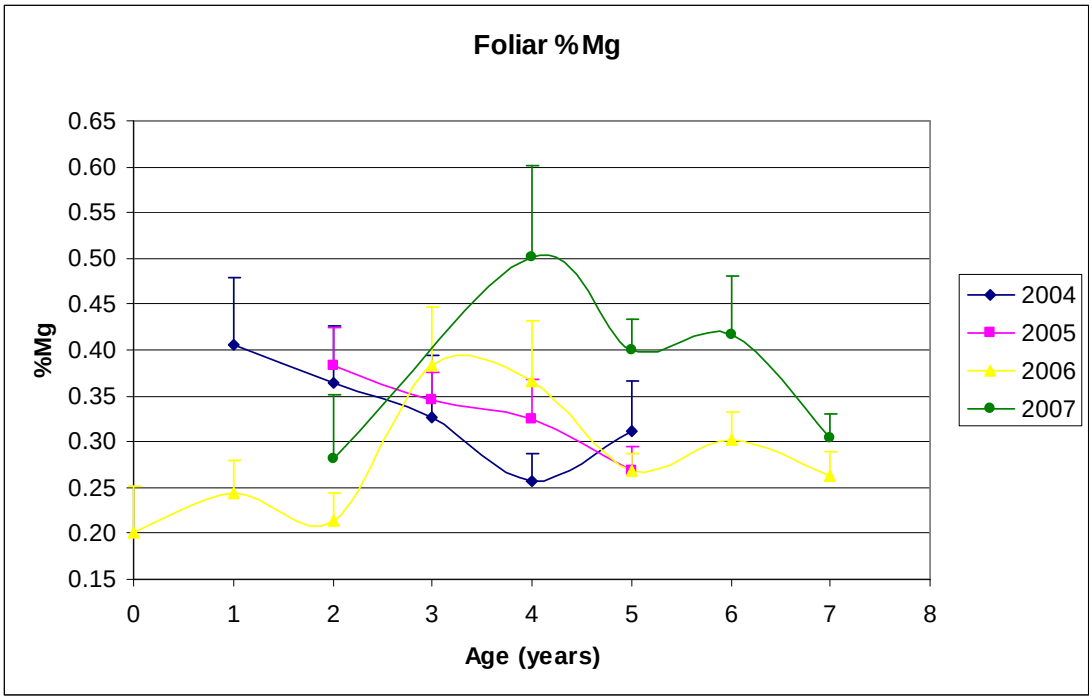

Figure 12. Foliar Magnesium Concentrations 2004-2007

Table 47. 2004-2007 ANOVA for Foliar Magnesium (%)

Age	Mean	SD	N	Tukey
0	0.20	0.05	5	A
1	0.35	0.10	15	B
2	0.32	0.08	24	B
3	0.35	0.06	19	B
4	0.34	0.11	24	B
5	0.31	0.06	24	B
6	0.36	0.08	10	B
7	0.28	0.04	9	B

Means with the same letter are not significantly different

Sulfur

The graphs for yearly mean foliar sulfur are found in Figure 13. The foliar sulfur data from 2004-2007 were pooled and analyzed. Log transformation of the data improved the graphs of the residuals and the decrease in the amount of skew and kurtosis. Thus, log transformed data was used to generate an ANOVA. The results of an ANOVA with Tukey adjustment are found in Table 48. The results indicate that the age 1 trees have significantly lower foliar sulfur levels than all other ages

except the age 4 trees. The probable explanation for this is that the trees use up any stores of sulfur as they work to establish their root systems between the time of planting and the time they reach age 2. Looking at the years 2006 and 2007, there appears to be a trend of lower foliar sulfur level in ages 3 or 4. Since rainfall is a major source of sulfur, it is possible that these lower levels may be related to the lack of rainfall at ERCO during the growing season in these years. However, since the biosolids also supply sulfur this may also be a reflection of difficulty converting the sulfur present in the biosolids into a form useable by the tree. It is also important to note that with the exception of ages 4 and 5 in 2005, the mean foliar sulfur levels for all other trees observed in this study fall below the ideal foliar sulfur concentration of 0.50% that is indicative of poplar trees with adequate nutrition (FAO, 1980).

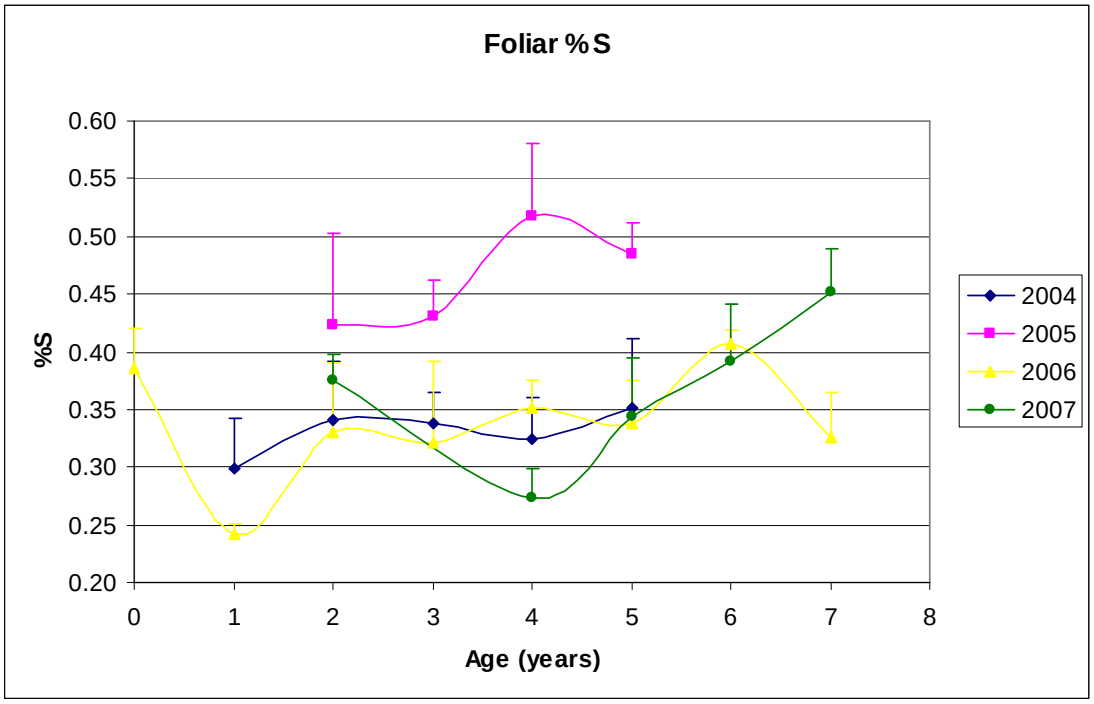

Figure 13. Foliar Sulfur Concentrations 2004-2007

Table 48. 2004-2007 ANOVA for Foliar Sulfur (%)

Age	Mean	SD	N	Tukey
0	0.39	0.03	5	A
1	0.28	0.05	15	B
2	0.36	0.06	24	A
3	0.35	0.06	19	A
4	0.35	0.09	24	A, B
5	0.37	0.07	24	A
6	0.40	0.03	10	A
7	0.38	0.08	9	A

Means with the same letter are not significantly different

Iron

The graphs for the yearly means of foliar iron are found in Figure 14. An evaluation of the pooled foliar iron data for 2004-2007 indicated that a transformation was necessary, with the graphs of the residuals indicating that log transformation was appropriate. Compared to untransformed data, log transformation decreased the amount of skew and kurtosis while improving the Shapiro-Wilk test statistic from 0.8325 to 0.9737. However, log transformation also resulted in a decrease in the p-value of the Levene's HOV test to 0.0180 from 0.0581. Keeping these results in mind, log transformed data were used to generate an ANOVA with Tukey adjustment. The results of the ANOVA, presented in Table 49, show that over the course of the study the age 1 trees had significantly higher foliar iron levels than trees ages 0 and 6. The foliar iron concentration of age 6 trees was also significantly lower than age 2 trees. While no other comparison were significant, the general trend appears to be a decrease in foliar iron levels as the trees grow older The low levels of foliar iron observed in this study indicate that iron availability to the trees is a cause for concern, and the probable cause for the decrease in foliar iron levels is most likely related to competition with other elements for sorption sites in the root zone.

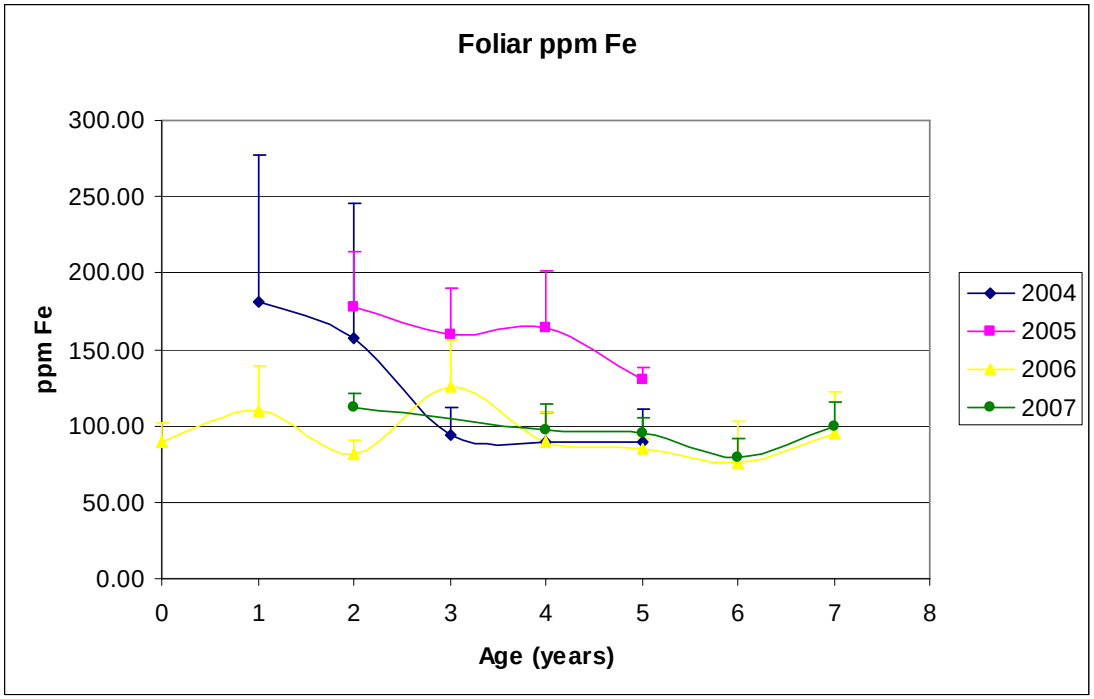

Figure 14. Foliar Iron Concentrations 2004-2007

Table 49. 2004-2007 ANOVA for Foliar Iron (ppm)

Age	Mean	SD	N	Tukey
0	90.00	12.00	5	A, B
1	157.50	86.22	15	C
2	135.67	67.35	24	B, C
3	116.31	35.03	19	A, B, C
4	103.20	35.03	24	A, B, C
5	96.65	21.31	24	A, B, C
6	77.40	20.07	10	A
7	97.89	22.16	9	A, B, C

Means with the same letter are not significantly different

Copper

Foliar copper data for 2004-2007 was pooled and analyzed. Mean yearly foliar data were graphed and presented in Figure 15. Data transformations did not result in an improvement of any of test metrics. In fact, the p-value for the Levene's HOV test statistic became problematic when log and square root transformations were applied. Thus, untransformed pooled data were used in the ANOVA. The results of the ANOVA with a Tukey adjustment are shown in Table 50. The results

indicate that the only significant difference is between trees of ages 0 and 1. A possible explanation for this is that the tree depletes any reserves that it has as it works to establish its root system. Since copper is taken up by trees at the root tips, the lack of an established root system hinders the uptake of copper resulting in lower foliar copper levels. The roots system becomes much more established once the tree reaches age 2. This difference may also be another example of the impact of limited sample size since the age 0 trees were only sampled in 2006.

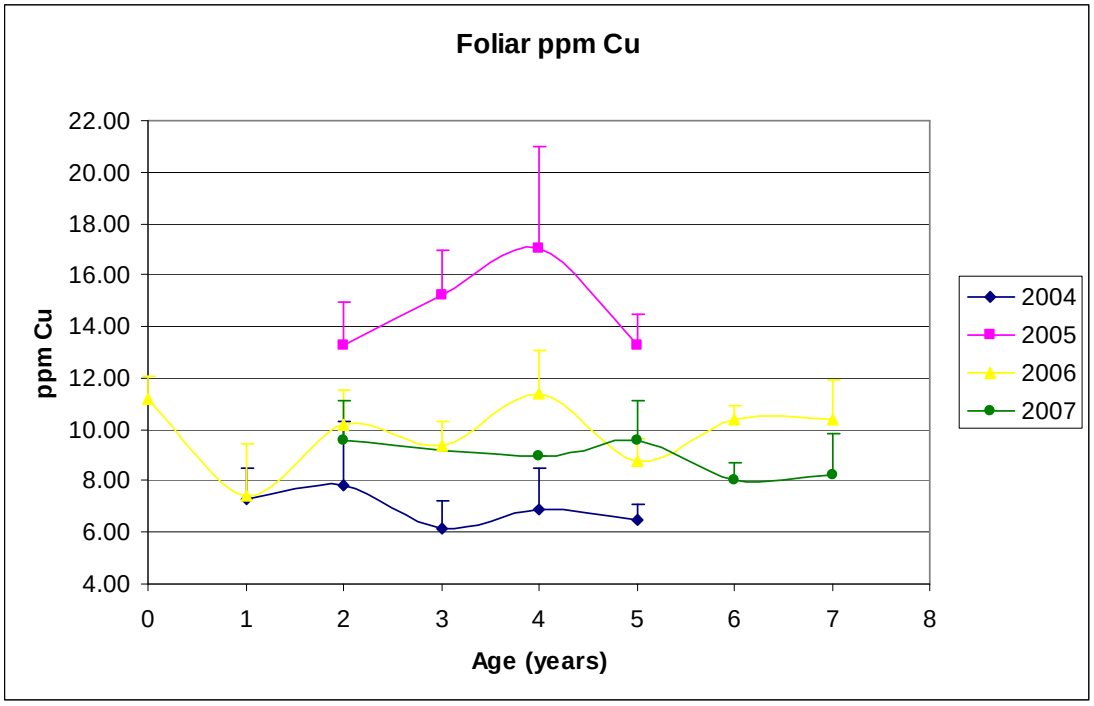

Figure 15. Foliar Copper Concentrations 2004-2007

Table 50. 2004-2007 ANOVA for Foliar Copper (ppm)

Age	Mean	SD	N	Tukey
0	11.20	0.84	5	A
1	7.35	1.49	15	B
2	9.61	2.67	24	A, B
3	8.93	3.80	19	A, B
4	9.95	4.13	24	A, B
5	8.75	2.59	24	A, B
6	9.20	1.40	10	A, B
7	9.56	1.81	9	A, B

Means with the same letter are not significantly different

Manganese

Foliar manganese means were graphed and presented in Figure 16. The graph of the residuals for the pooled 2004-2007 foliar manganese data suggested that a log transformation was needed. The log transformation also improved the Shapiro-Wilk test statistic value from 0.8289 to 0.9934. While log transformation lowered the p-value of the Levene's HOV test from 0.8339 to 0.0557, the p-value of the log transformed data was still greater than the 0.50 needed for the assumption of equal variances. The results of an ANOVA with a Tukey adjustment on log transformed data are presented in Table 51. The results indicate that the only significant differences in foliar manganese over the 2004-2007 period is that age 0 trees have significantly lower levels of foliar manganese than age 6 trees. While trees ages 3 and 6 have similar mean foliar manganese levels, the standard deviation for the age 3 trees are roughly three times greater than the standard deviation for the age 6 trees. The end result is that the age 3 trees are not significantly different than the age 0 trees while the age 6 trees are significantly different than the age 0 trees. The differences between the age 0 trees and the age 6 trees are most likely a result of the presence of a fully developed root system in older trees that is better able to access sources of manganese in the soil profile. Limited sample size may also play a role in this difference since the age 0 trees were only sampled in 2006. As previously mentioned, soil tests are a more appropriate method of determining plant manganese status (Altland, 2006).

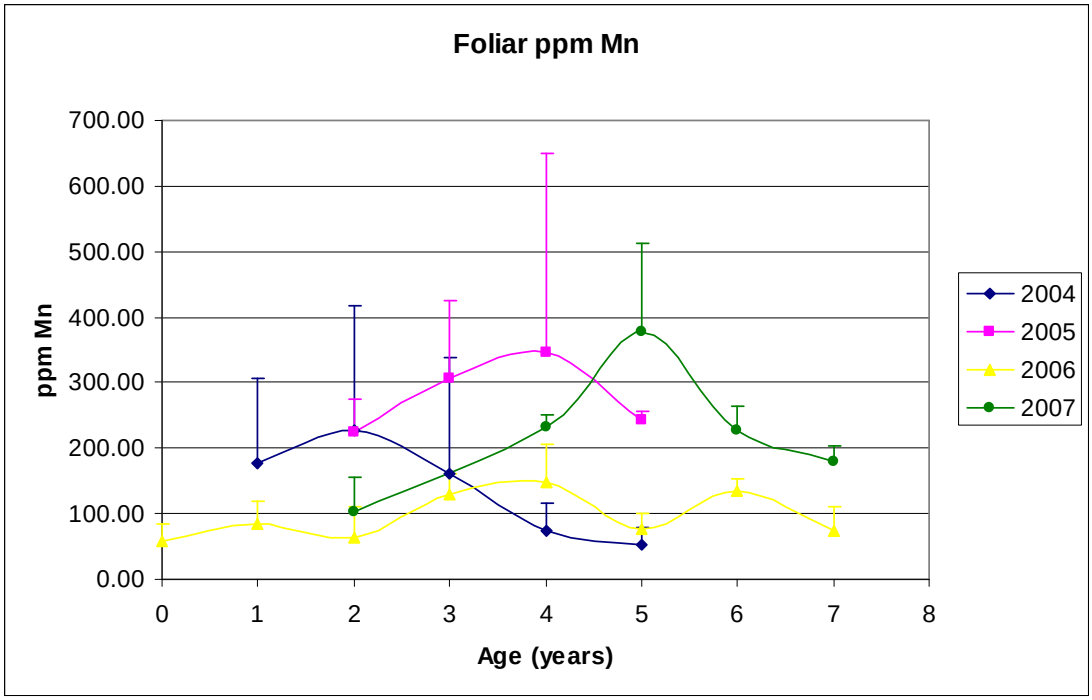

Figure 16. Foliar Manganese Concentrations for 2004-2007

Table 51. 2004-2007 ANOVA for Foliar Manganese (ppm)

Age	Mean	SD	N	Tukey
0	58.60	24.72	5	A
1	146.56	113.66	15	A, B
2	167.16	143.72	24	A, B
3	182.94	151.21	19	A, B
4	167.36	154.21	24	A, B
5	157.21	147.02	24	A, B
6	180.80	57.10	10	B
7	121.11	63.09	9	A, B

Means with the same letter are not significantly different

Zinc

Foliar zinc data from 2004-2007 were pooled together and analyzed. Figure 17 shows the graphs of the yearly means. The data were log transformed based on the graphs of the residuals. Log transformation also minimized the skew and the kurtosis while increasing the value of the Shapiro-Wilk test statistic to a very satisfactory 0.9932. The results of an ANOVA with Tukey adjustment on log transformed data are found in Table 52. These results indicate that there are no

significant differences between the different ages of trees over the course of the study. The higher mean for the age 1 trees is a result of the data from 2004. In 2004, 3 of the 10 samples had foliar zinc levels in excess of 155 ppm. Typical foliar zinc levels range from 15-100 ppm. The higher levels of zinc found in these three samples may be a result of the previous use of biosolids. However, the lack of any other observations of elevated zinc foliar concentrations seems to indicate that this was a spatially and temporally isolated event. It should also be noted that poplar trees are a species known to accumulate zinc and that this may also confound foliar analysis (Landis and van Steenis, 1998b).

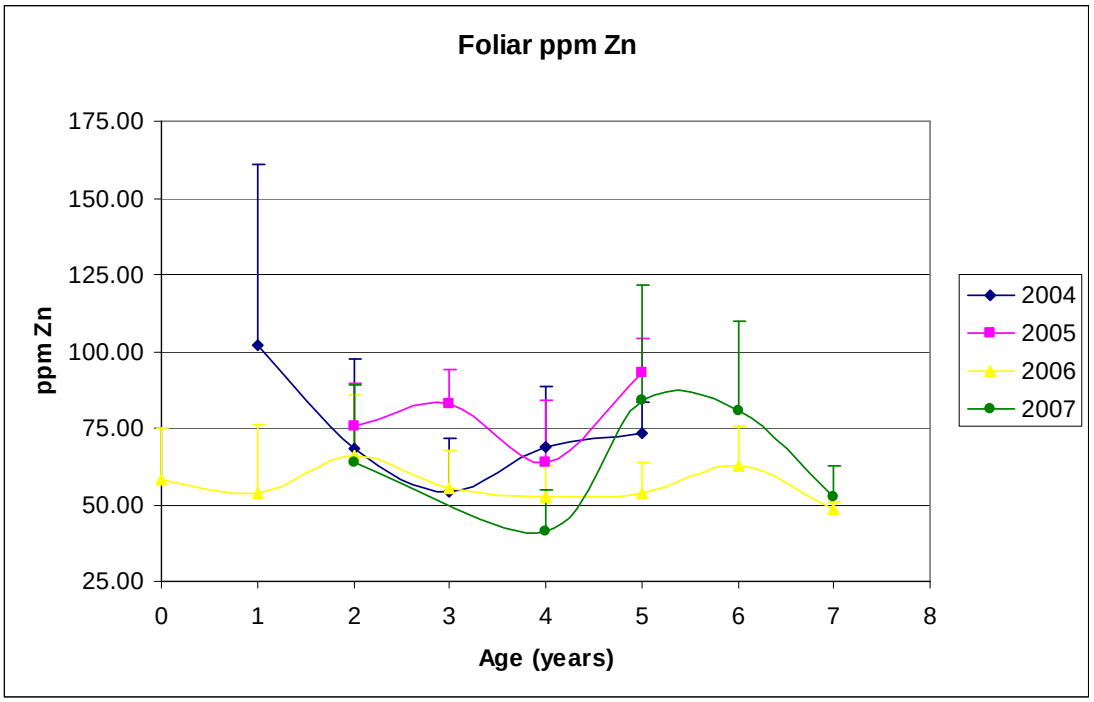

Figure 17. Foliar Zinc Concentrations 2004-2007

Table 52. 2004-2007 ANOVA for Foliar Zinc (ppm)

Age	Mean	SD	N	Tukey
0	58.20	17.06	5	A
1	85.79	54.36	15	A
2	68.03	23.74	24	A
3	60.73	18.55	19	A
4	58.88	19.52	24	A
5	74.82	22.28	24	A
6	71.70	23.26	10	A
7	50.56	7.76	9	A

Means with the same letter are not significantly different

Boron

Foliar boron data from all years, 2005-2007, were pooled together and analyzed. Graphs of the means by year are found in Figure 18. The data was log transformed. The log transformed Levene's HOV test statistic p-value of 0.0101, the best of any transformation tried, still shows unequal variances within the means. The log transformation improved the graphs of the residuals. The Shapiro-Wilk test statistic value of 0.9840 of the log transformed data was the best of all the transformations and it also shows adequate normality of distribution for treatment means. Thus, the log transformed data were used in the ANOVA. The results of an ANOVA using a Tukey adjustment are presented in Table 53. The data indicate that foliar boron appears to peak in trees at age 6. The data also seem to indicate that significant differences between older and young trees are most evident once trees reach ages 4 or 5. It is also possible that the variations in the foliar boron for trees ages 5, 6, and 7 might be a result of the differences in the time of collection. Leaves were collected at the end of the third week in August for 2007 compared to the end of the first week/start of the second week in August for 2006. While the collection times for leaves fit within the range needed for stable leaf nitrogen values, it is quite possible that boron levels within the leaves during the latter weeks of August may be

fluctuating. Additionally, boron uptake is impacted by water availability. It is conceivable that the variation in the amount of water available to the trees throughout the different growing seasons had an impact on the fluctuations of foliar boron levels seen in older trees between the different years. One final note: all of the foliar boron levels were well below the toxic level of 200 ppm and above the general minimum level of 20 ppm throughout the study.

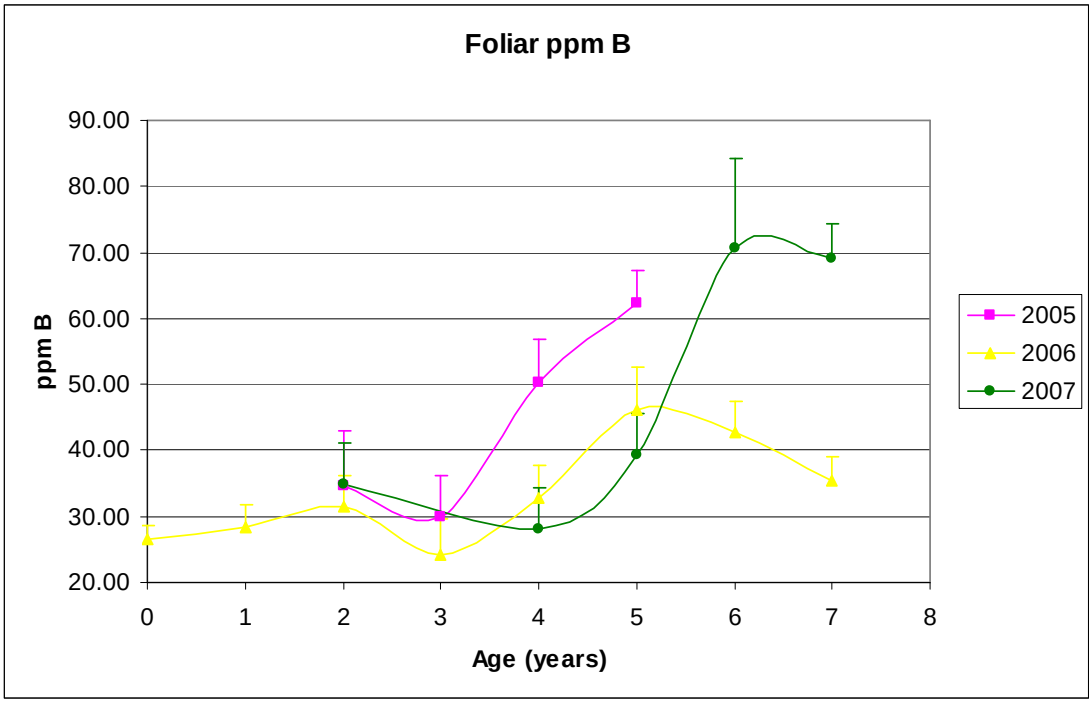

Figure 18. Foliar Boron Concentrations for 2005-2007

Table 53. 2005-2007 ANOVA for Foliar Boron (ppm)

Age	Mean	SD	N	Tukey
0	26.6	2.07	5	A
1	28.4	3.36	5	A
2	33.57	6.25	14	A,B
3	26.78	6.26	9	A
4	36.07	11.02	14	A,B
5	48.36	11.10	14	B,C
6	56.7	17.68	10	C
7	49.89	17.66	9	B,C

Means with the same letter are not significantly different

Sodium

Foliar sodium data from 2005-2007 were pooled together for analysis. Graphs of the yearly means are found in Figure 19. Transformed data did not improve upon the untransformed values for Levene's HOV test (0.2638) or the Shapiro-Wilk test statistic (0.8398). The graphs were also unchanged. The low value of the Shapiro-Wilk statistic is probably impacted by the lack of replication and by the small amount of variation found in the samples, thus affecting correlation to the ideal normal distribution. Keeping the limitations in mind, untransformed data were used in the ANOVA. The ANOVA results using a Tukey adjustment are presented in Table 54. The data suggests that foliar sodium levels decrease as the tree ages, with a significant difference between younger and older trees becoming evident around age 3. This suggests that the tree has become established and the root system has grown large enough by age 4 to overcome some of the impacts of water stress found in the ERCO poplar plantation by fully utilizing the water storage potential of the biosolids. This is especially important for tree survival given that the years 2006 and 2007 experienced lower amounts of rainfall during the growing season, evident in the lower foliar sodium values found in all ages for 2005. An argument may also be made that once the trees end the seventh year of growth they have exhausted the available water found in the biosolids. This may explain the increase, though not statistically significant, in the foliar sodium levels of age 7 trees when compared to younger ages of trees. It should also be noted that a majority of the trees tested had foliar sodium concentrations at the limits of detection (0.010%). This is indicates that salinity does not appear to be an issue at the ERCO tree plantation.

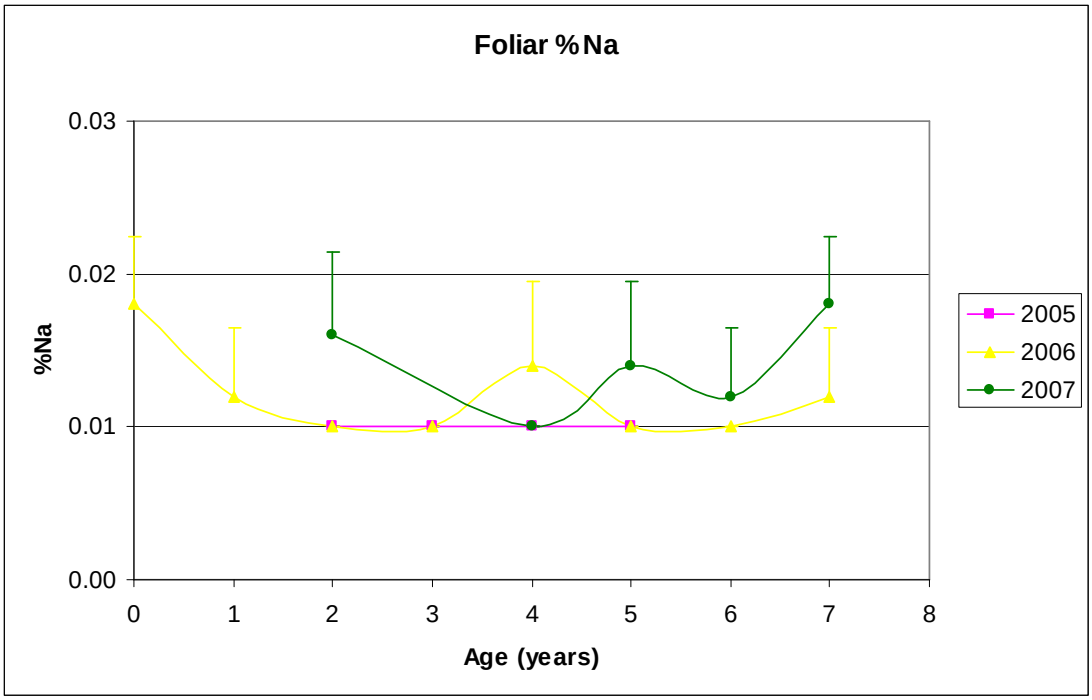

Figure 19. Foliar Sodium Concentrations for 2005-2007

Table 54. 2005-2007 ANOVA for Foliar Sodium (%)

Age	Mean	SD	N	Tukey
0	0.018	0.004	5	A
1	0.012	0.004	5	A,B
2	0.014	0.005	14	A,B
3	0.010	0.000	8	B
4	0.011	0.004	14	B
5	0.011	0.004	14	B
6	0.011	0.003	10	B
7	0.014	0.005	9	A,B

Means with the same letter are not significantly different

Aluminum

The foliar aluminum data sets for 2005-2007 were pooled together and analyzed. Yearly means were calculated and graphed (Figure 20). The graphs of the residuals suggested that a log transformation was needed. Log transformation greatly improved the graphs of the residuals and the value of the Shapiro-Wilk test (0.9753). Though the p-value for Levene's HOV test (0.0677) was lower than that of the untransformed data, it was still greater than 0.05 needed to indicate equal variances.

The results of an ANOVA with a Tukey adjustment on log transformed data are presented in Table 55. These results indicated that there was no significant difference among the different ages of trees in foliar aluminum concentrations over the course of the study.

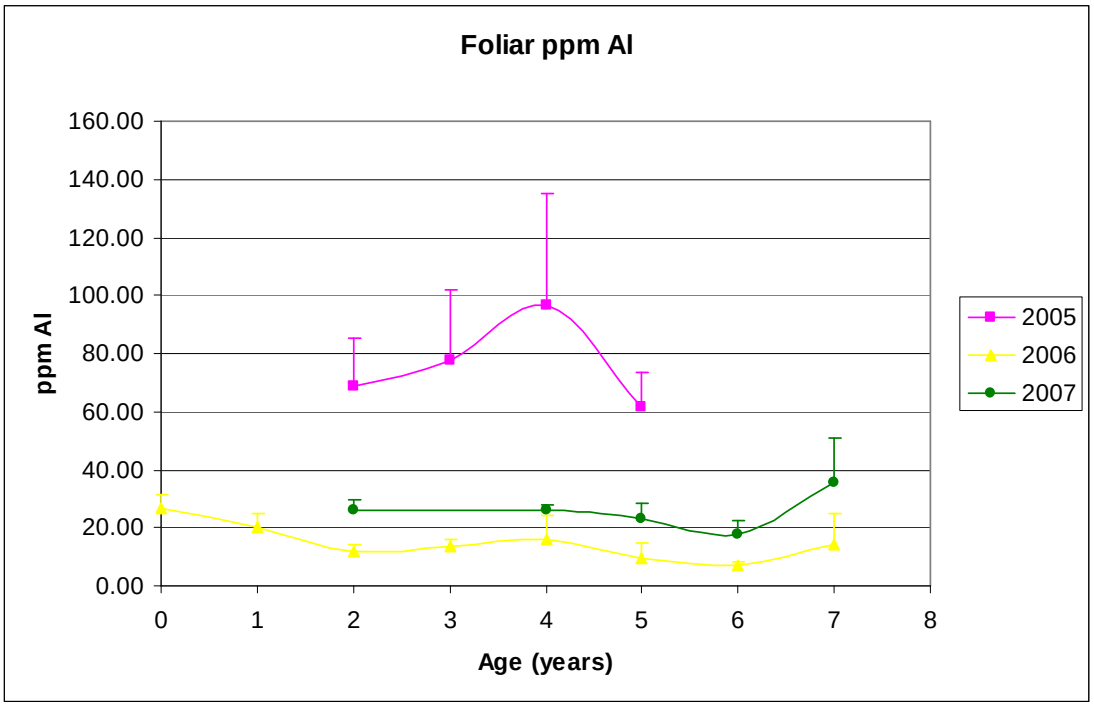

Figure 20. Foliar Aluminum Concentrations for 2005-2007

Table 55. 2005-2007 ANOVA for Foliar Aluminum (ppm)

Age	Mean	SD	N	Tukey
0	26.80	4.87	5	A
1	20.40	4.77	5	A
2	33.14	25.49	14	A
3	42.11	36.86	9	A
4	42.79	40.42	14	A
5	29.29	23.27	14	A
6	12.50	6.64	10	A
7	24.44	17.72	9	A

Means with the same letter are not significantly different

Modeling Results

General Comments

Models were constructed of the 2004-2007 data for each nutrient using the PLS2 option in Unscrambler 9.1. Full cross-validation models were constructed using the pooled 2004-2007 foliar nutrient data. The full cross-validation models also utilized the Marten's Uncertainty Test in determining which spectral regions were significant in the model. Test set models were constructed using the 2004-2006 data for calibration and 2007 data for validation. Model performance for both the calibration and validation portion of the models was evaluated using the number of principal components (PCs), the slope of the regression line, the root mean squared error (RMSE), and the r-squared. The best performing models would ideally have fewer PCs, a regression slope of 1 or -1 for the predicted vs. measured values, low values for RMSE and an r-squared value near 1. The number of PCs used in each model corresponded to the number of PCs that resulted in the lowest global variance. Wavelengths were transformed using Savitzky-Golay Smoothing algorithms alone or in addition to 1^{st} and 2^{nd} derivatives on the truncated or visible spectra.

Carbon

Foliar carbon and spectral reflectance data were used to generate a full cross-validation model. The results, found in Table 56, indicate a poor relationship between spectral data and foliar carbon concentrations. This is evident in the low r-squared and slope values for both the calibration and validation portions of the model.

Table 56. Full Cross-Validation Model based on Untransformed Truncated Spectra and Untransformed Carbon Data

	# of PCs	Slope	RMSE	r-squared
Calibration	3	0.2827	0.5671	0.2827
Validation	3	0.1754	0.6397	0.1168

A test set model for foliar carbon was also evaluated, using 2004 data for calibration and 2005 data for validation. The results of this model, found in Table 57, indicate the lack of correlation between spectral data and foliar carbon concentrations. The r-squared values and the slopes are both very small, and these are indicative of little or no correlation between spectral data and foliar carbon concentrations. Overall, the results of these models indicate that there is no correlation between spectral data and foliar carbon concentrations

Table 57. Test Set Model base on Untransformed Truncated Spectra and Untransformed Carbon Data

	# of PCs	Slope	RMSE	r-squared
Calibration	2	0.0725	0.5744	0.0725
Validation	2	-0.0894	0.9165	0.1177

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Hydrogen

Full cross-validation was the only model generated for predicting foliar hydrogen levels since data for foliar hydrogen was only available for the year 2004. The results, found in Table 58, indicate that there appears to be a strong correlation between spectral data and foliar hydrogen concentrations. This is evident in the high values for slope and for r-squared in both the calibration and validation portions of the model. These results indicate that further research into the relationship between foliar hydrogen and spectral reflectance is warranted.

Table 58. Full Cross-Validation Model based on Untransformed Truncated Spectra and Untransformed Hydrogen Data

	# of PCs	Slope	RMSE	r-squared
Calibration	5	0.8475	0.1086	0.8411
Validation	5	0.7516	0.1548	0.6838

Nitrogen

The best results obtained from full cross-validation testing on foliar nitrogen data are presented in Table 59. This model utilized the Savitzky-Golay smoothing transformation on the visible and red edge wavelengths of the spectrum. This model had the best combination of slope, RMSE and r-squared values compared to other models evaluated. Specifically, the r-squared values of this model were among the highest of any nutrient evaluated during this study. The values for both the slope and for r-squared indicate that there appears to be a correlation between foliar nitrogen concentrations and spectral data.

Table 59. Full Cross-Validation Model based on Savitzky-Golay Smoothing transformation of the Visible and Red Edge Spectra and Untransformed Nitrogen Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.6585	0.2612	0.6585
Validation	12	0.6141	0.3018	0.5512

The best results for the test set model for foliar nitrogen, using 2004-2006 data for calibration and 2007 data for validation, were obtained using Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative transformations on truncated wavelengths. These results are presented in Table 60. The validation portion of this model, in particular the near zero values for the slope and for r-squared, indicate a poor relationship between spectral and nutrient data in the model. The results of the calibration part of the model are only marginally better. Overall, the lack of adequate values in the test set model indicates that there appears to be a poor predictive correlation between spectral data and foliar nitrogen when applied to this study. The differences between the full cross-validation model and the test set model are most likely a case of over-fitting by the cross-validation model. While the results of the test set model are disappointing, the results of the full cross-validation model do

indicate that further study into the predictive ability of spectral data on foliar nitrogen levels is warranted. Additionally, the results of a test set model utilizing a random selection of 20% of the samples, found in Table 61, showed promise in predicting foliar nitrogen concentrations. The differences in the test set models indicate the importance of the selection of samples used to generate test set. It is plausible that the use of the 2007 data alone to generate a test set fails to capture the necessary variation needed in the generation of a predictive model that adequately predicts foliar nitrogen concentrations.

Table 60. Test Set Model based on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformations of Truncated Spectra and Untransformed Nitrogen Data

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.1967	0.4145	0.1966
Validation	4	0.0677	0.4290	0.0428

Table 61. Test Set Model with Random 20% Test Set based Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformations of Visible and Red Edge Spectra and Untransformed Nitrogen Data

	# of PCs	Slope	RMSE	r-squared
Calibration	6	0.5080	0.324	0.5080
Validation	6	-5570	0.3333	0.5102

Phosphorus

The best results obtained from full cross-validation testing on foliar phosphorus data are presented in Table 62. This model utilized the Savitzky-Golay smoothing transformation on the visible and red edge wavelengths of the spectrum. The calibration segment of the model has very satisfactory values for both slope and r-squared. However, the r-squared value for the validation portion of the model of 0.2207 is much lower than the 0.6897 r-squared value of the calibration portion. This is indicative of problems with the model.

Table 62. Full Cross-Validation Model based on Savitzky-Golay Smoothing Transformation of Visible and Red Edge Spectra and Untransformed Phosphorus Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.6898	0.0529	0.6897
Validation	12	0.6318	0.0608	0.2207

The best results for the foliar phosphorus test set model, using 2004-2006 data for calibration and 2007 data for validation, were obtained using Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative transformations on truncated wavelengths. These results, found in Table 63, indicate that, based on the values of the slope and r-squared, there is a poor relationship between spectral data and foliar data. While the slope of the validation segment is much better, it comes at the expense of an increase in the RMSE. The promise of the cross-validation model for predicting foliar phosphorus did not hold up in the test set model. These differences are most likely the result of over-fitting. The r-squared values of the cross-validation model are interesting enough to warrant further research into the predictive abilities of spectral data for foliar phosphorus concentrations.

Table 63. Test Set Model based on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformations on Truncated Spectra and Untransformed Phosphorus Data.

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.3082	0.0706	0.3081
Validation	4	0.5614	0.1562	0.2412

Potassium

The Savitzky-Golay smoothing transformation on the visible and red edge spectra provided the best results for the full cross-validation model for foliar potassium. The results of the model, found in Table 64, indicate the possibility of a strong relationship between spectral data and foliar potassium concentrations. This is evident in the values for the slope and for r-squared.

Table 64. Full Cross-Validation Model based on Savitzky-Golay Smoothing Transformation on the Visible and Red Edge Spectra and Untransformed Potassium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.6826	0.4655	0.6826
Validation	12	0.6272	0.5339	0.5855

The Savitzky-Golay smoothing and the Savitzky-Golay 2nd derivative transformations on truncated spectra using the 2004-2006 data for calibration and the 2007 data for validation provided the best results for the test set model for foliar potassium. The result of the model, found in Table 65, show a marked decrease in the predictive ability when compared to the full cross-validation model. The validation part of the test set model has larger values for the slope and r-squared at the expense of a larger value for RMSE compared to the calibration portion of the model. It is plausible that the larger RMSE values explain the larger values for slope and r-squared. While the full cross-validation model indicates a strong correlation between foliar potassium concentrations and spectral data, a comparison with the values for the slope and for r-squared from the results of the test set model indicate that the correlation appears to be much weaker. However, the strength of the cross-validation model indicates that future study of the prediction of foliar potassium levels using spectral data is justified.

Table 65. Test Set Model base on Savitzky-Golay Smoothing and Savitzky-Golay 1st Derivative Transformed Truncated Spectra and Untransformed Potassium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.1262	0.7278	0.1261
Validation	1	0.2011	1.2008	0.2036

Calcium

The best results obtained from full cross-validation testing on foliar calcium data are presented in Table 66. This model utilized the Visible and Red Edge

wavelengths of the spectrum. This model had the best combination of slope, RMSE and r-squared values compared to other models evaluated.

Table 66. Full Cross-Validation Model on based on Untransformed Visible and Red Edge Spectra and Untransformed Calcium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	10	0.5733	0.2886	0.5734
Validation	10	0.5194	0.3218	0.4739

The best results for foliar calcium for the test set model, using 2004-2006 data for calibration and 2007 data for validation, were obtained using Savitzky-Golay 1st derivative transformations on truncated wavelengths. These results are presented in Table 67. Both the calibration and validation portions of the model, especially the near zero values for the slope, indicate a poor relationship between spectral and nutrient data. The differences between the full cross-validation model and the test set model are most likely a case of over-fitting by the cross-validation model. It does not appear that the use of spectral reflectance data is a viable predictive methodology for estimating foliar calcium levels.

Table 67. Test Set Model based on 1st Derivative Transformed Truncated Wavelengths and Untransformed Calcium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.1620	0.2616	0.1620
Validation	1	0.0226	0.9017	0.2638

Magnesium

The best results of the full cross-validation model for foliar magnesium, found in Table 68, were found using Savitzky-Golay smoothing transformations on the visible and red edge wavelengths. These results indicate that there appears to be a weak relationship between spectral reflectance and foliar magnesium concentrations.

Table 68. Full Cross-Validation Model based on Savitzky-Golay Smoothing Transformations on Visible and Red Edge Spectra and Untransformed Magnesium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.3910	0.0666	0.3910
Validation	12	0.3029	0.0759	0.2303

The test set model results for foliar magnesium, using 2004-2006 data for calibration and 2007 data for validation are found in Table 69. These were obtained using Savitzky-Golay smoothing and Savitzky-Golay 1st derivative transformations on truncated spectra. The near zero values for the slope in the calibration part of the model and for both the slope and for r-squared in the validation part of this model indicate that there is no relationship between foliar magnesium concentrations and spectral data. While the r-squared of the validation part of the model did increase when compared to the calibration part of the model, the fact that the slope for the validation part of the model is still near zero indicates no correlation between variables. The differences between the two models are most likely a result of over-fitting by the full cross-validation model.

Table 69. Test Set Model based on Savitzky-Golay Smoothing and Savitzky-Golay 1st Derivative Transformations of Truncated Spectra and Untransformed Magnesium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.0893	0.0726	0.0893
Validation	1	0.0272	0.1243	0.2320

Sulfur

For foliar sulfur the best results of the full cross-validation model were obtaining using the visible and red edge spectra transformed using Savitzky-Golay smoothing. These results, presented in Table 70, show that there is evidence of a relationship between spectral data and foliar sulfur concentrations. The decrease in the r-squared value of the validation portion of the model when compared to the

calibration portion of the model is cause for concern about the overall predictive capabilities of this model.

Table 70. Full Cross-Validation Model based on Savitzky-Golay Smoothing Transformed Visible and Red Edge Spectra and Untransformed Sulfur Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.4595	0.0522	0.4550
Validation	12	0.3459	0.0620	0.2600

Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative transformations on the visible and red edge spectra provided the best overall results in the test set model for foliar sulfur. The model was generated using 2004-2006 data for calibration and 2007 data for validation. Found in Table 71, the results of the test set model show a marked decrease from the results of the cross-validation model in Table 70. The correlation between spectral data and foliar sulfur concentrations appears to be much smaller in the test set model. The differences between the two models may be due to over-fitting in the cross-validation model. The values of the test set model indicate that prediction of foliar sulfur concentrations by spectral data does not appear to be a viable methodology at this time.

Table 71. Test Set Model based on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformed Visible and Red Edge Spectra and Untransformed Sulfur Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.1272	0.0665	0.1272
Validation	1	0.0891	0.3125	0.0976

Iron

For foliar iron the best results of the full cross-validation model were obtained by transforming truncated spectra using Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative. The results of the model, found in Table 72, show that there may be a weak relationship between spectral reflectance and foliar iron data. This is evident in the values for the slope and the r-squared.

Table 72. Full Cross-Validation Model base on Truncated Spectra transformed by Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative and Untransformed Iron Data

	# of PCs	Slope	RMSE	r-squared
Calibration	5	0.3603	41.2382	0.3604
Validation	5	0.2662	46.1812	0.2118

For foliar iron the test set model using 2004-2006 data for calibration and 2007 data for validation, the best results were obtained by using a Savitzky-Golay 2nd derivative transformation on the visible and red edge wavelengths of the spectrum. Found in Table 73, the results of the test set model indicate a poor relationship between spectra and foliar iron. While the slopes are greater than zero, they are still indicative of a lack of correlation between the two variables. Combined with the low values for r-squared, these factors indicate that the data does not support using spectral reflectance for the prediction of foliar iron.

Table 73. Test Set Model based on Savitzky-Golay 2nd Derivative Transformed Visible and Red Edge Spectra and Untransformed Iron Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.1473	51.5801	0.1473
Validation	1	0.1024	27.2127	0.1108

Copper

Full cross-validation on the 2004-2007 foliar copper data suggested that there may be a relationship between reflectance spectra and foliar copper concentrations. The results of the model, found in Table 74, showed that there were acceptable values for slope and r-squared in the calibration portion of the model. While these values were lower in the validation portion of the model, they still demonstrated that there may be a relationship between spectral data and foliar copper concentrations.

Table 74. Full Cross-Validation Model base on Savitzky-Golay Smoothing Transformed Visible and Red Edge Spectra and Untransformed Copper Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.5724	1.9412	0.5727
Validation	12	0.4709	2.3458	0.3918

The best results for foliar copper in the test set model was achieved by using Savitzky-Golay Smoothing and Savitzky-Golay 2nd derivative transformations on truncated spectra. The results of the model, presented in Table 75, show that there is poor correlation between the calibration and validation segments. The calibration shows acceptable values for slope and r-squared. However, the validation segment has values of practically zero for slope and r-squared, and this is indicative of a lack of correlation between spectral data and foliar data. Thus, the model does not accurately predict the 2007 data.

Table 75. Test Set Model base on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformations on Truncated Spectra and Untransformed Copper Data

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.5297	2.2145	0.5296
Validation	4	-0.0273	1.6122	0.0024

There are a number of possible explanations for the differences between calibration and validation in the test set and the difference between the test set and the full cross-validation models. First, it is possible that the model is not accurate. Full cross-validation often results in over-fitting, and this would make a model appear to adequately predict values when in fact it is does not. This inability to predict would be uncovered when using the test set model. Second, the 2007 dataset for foliar copper may represent some spectral anomaly that the model is unable to account for. This is unlikely since the anomaly should be discernable in the graph of the regression coefficients. Next, it is possible that other factors might play a role in confounding the model. Copper uptake is affected by water, and the amount of rainfall at the ERCO tree farm was quite different over the four years of the study. This in turn may affect the amounts of aluminum and iron available to compete with copper for uptake by the roots. Finally, it is entirely possible that it is just not

possible to predict foliar copper levels using spectral data. Interactions with other elements coupled with minute foliar concentrations to begin with may present an obstacle that spectral modeling is unable to overcome. These problems might be exacerbated by the timing of foliar sample collection compounded with small sample sizes. Foliar sampling traditionally focuses on nitrogen and phosphorus, and the timing of sampling corresponds to when these nutrients are stable. It is possible that copper levels in the leaves are not stable at this time. Additionally, there is debate about whether the number of samples traditionally collected for foliar analysis is adequate to accurately measure the concentration of foliar metals. The thought is that the small concentrations of these metals suggest that more samples are needed in order to get a complete picture of foliar health for the area or species of interest. Finally, a preliminary evaluation of a test set model utilizing a 20% random sample selection from the pooled 2004-2007 data appears to indicate that prediction of foliar copper levels from reflectance data is possible.

Manganese

The best results of the full cross-validation model on foliar manganese were obtained using untransformed visible and red edge spectra. The results of the model, found in Table 76 show that there may be a weak relationship between spectral reflectance and foliar iron data. This is evident in the values for the slope and the r-squared. However, the RMSE for the model is quite large and presents a cause for concern.

Table 76. Full Cross-Validation Model based on Untransformed Visible and Red Edge Spectra and Untransformed Manganese Data

	# of PCs	Slope	RMSE	r-squared
Calibration	10	0.2984	110.4250	0.2984
Validation	10	0.2164	122.0835	0.1614

For the test set model for foliar manganese using 2004-2006 data for calibration and 2007 data for validation, the best results were obtained by using Savitzky-Golay smoothing and the Savitzky-Golay 2nd derivative transformations on truncated spectra. Found in Table 77, the results of the test set model indicate contradictory results between the calibration and validation parts of the model. Similar to the results of the full cross-validation model, the values for slope and r-squared in the calibration portion of the test set model indicates that there appears to be a weak relationship between spectral data and foliar manganese concentrations. However, the practically zero values for slope and r-squared in the validation segment of the test set model indicates a lack of correlation between foliar data and spectral data. The combination of the lack of agreement between the calibration and validation parts of the test set model with the large RMSE values for both the cross-validation and test set models indicates that using spectral reflectance to predict foliar manganese levels is not a valid methodology.

Table 77. Test Set Model base on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformed Truncated Spectra and Untransformed Manganese Data

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.2368	114.9752	0.2367
Validation	4	0.0186	145.4731	0.0060

Zinc

Savitzky-Golay smoothing and Savitzky-Golay 1st derivative transformations on the visible and red edge spectra produced the best predictive results for foliar zinc concentrations. The results, found in Table 78, show some overall correlation

between spectral data and foliar zinc levels. The validation model does exhibit a lower amount of correlation between variables as seen by the lower values for both slope and r-squared.

Table 78. Full Cross-Validation Model based on Savitzky-Golay Smoothing and Savitzky-Golay 1st Derivative Transformations of Visible and Red Edge Spectra and Untransformed Zinc Data

	# of PCs	Slope	RMSE	r-squared
Calibration	6	0.3159	22.8695	0.3158
Validation	6	0.2018	25.5920	0.1566

The Savitzky-Golay 2nd derivative transformation on truncated spectra provided the best overall results in the test set model for foliar zinc. The model was generated using 2004-2006 data for calibration and 2007 data for validation. The results of the test set model, found in Table 79, show a marked decrease in r-squared values when compared to the results of the cross-validation model. However, the values for the slopes of the two models remain about the same, and this is indicative of a level of agreement between the two models. Both models indicate that there appear to be a correlation, albeit slight, between variables that would allow for the prediction foliar zinc levels using spectral data. One area of concern is the near zero value of r-squared. While the slope indicates that there is some level of correlation between predicted and measure values, the small r-squared is indicative of a lack of agreement between the model and the dataset. Since poplars do accumulate zinc, it is possible that this accumulation is interfering with the model.

Table 79. Test Set Model based on 2nd Derivative Transformation of Truncated Spectra and Untransformed Zinc Data

	# of PCs	Slope	RMSE	r-squared
Calibration	9	0.3894	21.4840	0.1047
Validation	9	0.2153	26.4536	0.0198

Boron

The best results obtained from full cross-validation testing on foliar boron data are presented in Table 80. The results were accomplished using a model that utilized Savitzky-Golay smoothing on the Visible and Red Edge wavelengths of the spectrum. This model had the best combination of slope, RMSE and r-squared values compared to other models evaluated.

Table 80. Full Cross-Validation Model based on Visible and Red Edge Spectrums with Savitzky-Golay Smoothing and Untransformed Boron Data

	# of PCs	Slope	RMSE	r-squared
Calibration	12	0.5049	10.8012	0.4892
Validation	12	0.4028	12.6712	0.3166

The best results for the test set model for foliar boron, using 2005-2006 data for calibration and 2007 data for validation, were obtained using Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative transformations on truncated wavelengths. These results are presented in Table 81. While the calibration portion of the model showed adequate predictive results, the slope and r-squared for the validation portion of the model was very small and is indicative of a poor relationship between predicted and measured data. The poor results in the validation portion of the model in all likelihood demonstrate that spectral analysis for measuring foliar boron levels is not a valid method.

Table 81. Test Set Model base on Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformed Truncated Spectra and Untransformed Boron Data

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.4028	8.7593	0.4187
Validation	4	0.0306	21.2904	0.0538

Sodium

A full cross-validation model for foliar sodium was generated using the data from 2005-2007. Savitzky-Golay 2nd derivative transformations on the Visible and

Red Edge wavebands yielded the best results. These results, found in Table 82, indicate that there is no correlation between spectral data and foliar sodium levels. This is especially evident in the small values for slope and r-squared found in the validation segment of the model. Finally, a preliminary evaluation of a test set model utilizing 20% random sample selection appears to indicate that prediction of foliar boron levels from reflectance data is possible.

Table 82. Full Cross-Validation Model base on Savitzky-Golay 2nd Derivative Transformed Visible plus Red Edge Spectra and Untransformed Sodium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	6	0.1637	0.0097	0.1604
Validation	6	-0.0310	0.0115	0.0075

A test set model for foliar sodium was generated using 2005-2006 data for calibration and 2007 data for validation. The best results were found using a Savitzky-Golay 2nd derivative transformation on truncated spectra. Similar to what was found using a full cross-validation model, the test set model results, found in Table 83, indicates that there is no relationship between spectral data and foliar sodium levels. Evidence of this is the fact that the r-squared values and the slope values are essentially zero. It does not appear that using spectral data to predict foliar sodium levels is a viable methodology.

Table 83. Test Set Model base on Savitzky-Golay 2nd Derivative Transformed Truncated Spectra and Untransformed Sodium Data

	# of PCs	Slope	RMSE	r-squared
Calibration	1	0.0754	0.0101	0.0628
Validation	1	-0.0003	0.0109	0.0231

Aluminum

Full cross-validation of the pooled foliar aluminum data from 2005-2007 suggested that untransformed truncated wavelengths would provide the best results for the prediction of foliar aluminum concentrations from spectral data. The cross-

validation model results are found in Table 84. The values for the slope and r-squared in the calibration part of the model were large enough to raise expectations on the predictive ability of the model.

Table 84. Full Cross-Validation Model based on Untransformed Truncated Wavelengths and Untransformed Aluminum Data

	# of PCs	Slope	RMSE	r-squared
Calibration	8	0.7587	14.6783	0.7135
Validation	8	0.5909	20.4855	0.4628

However, further analysis showed that the best results for foliar aluminum prediction were found by using truncated wavelengths with Savitzky-Golay smoothing and Savitzky-Golay 2nd derivative transformations applied to a test set model using 2005-2006 data for calibration and 2007 data for validation. These results are found in Table 85. Unfortunately, the predictive ability of the test set has a very low r-squared value of 0.1979. This was much lower than the initial validation r-squared value of 0.4628 found in the cross-validation model. This marked decrease in values is indicative of a poor predictive correlation between the calibration and test sets of the model. The values of both the slopes and r-squared in the calibration parts of the models indicates that further research into the relationship between spectral reflectance and foliar aluminum concentrations is warranted.

Table 85. Test Set Model based on Truncated Wavelengths with Savitzky-Golay Smoothing and Savitzky-Golay 2nd Derivative Transformations and Untransformed Aluminum Data

	# of PCs	Slope	RMSE	r-squared
Calibration	4	0.5992	20.3898	0.5907
Validation	4	0.4582	9.9041	0.1979

Leaf Water Potential

Full cross-validation of the water stress data for 2004 suggested that the Savitzky-Golay smoothing transformation on the visible and red edge wavelengths would provide the best results for the prediction of leaf water potential from spectral

data. The results of the model are found in Table 86, and they indicate that there appears to be a relationship between leaf water potential and spectral data. The values for the slope and r-squared in the calibration part of the model are quite large, demonstrating promise for the predictive ability of the model. However, this is tempered by the much lower values observed in the validation portion of the model.

Table 86. Full Cross-Validation Model based on Visible and Red Edge Spectra with Savitzky-Golay Smoothing on Untransformed Leaf Water Potential Data

	# of PCs	Slope	RMSE	r-squared
Calibration	8	0.7202	2.7377	0.7201
Validation	8	0.4459	4.3436	0.3341

Conclusions

Foliar Nutrients

Evaluation of the foliar data suggests that the age of the tree does have an effect on the concentration of certain foliar nutrients. Whether this is truly a result of the age of the tree is somewhat obscured by the conditions present at the ERCO site during the course of the study. The lack of adequate rainfall during the growing season for at least two years of the study is one possible explanation for the differences in the foliar nutrients observed, both during each year and between the different years. Lack of rainfall has a negative impact on the trees, affecting tree survival, growth and nutrient uptake. Each of these in turn influences the concentration of nutrients in the leaves. Additionally, one other key difference is the soil profile of the different sections. The makeup of the soil matrix in the different sections, assumed to be relatively similar, is in some areas actuality quite different. Not only are some of the sections quite different from each other, the variability within each section is decidedly marked. These differences in the soil profile may also play a role in tree survival, growth and nutrient uptake. Finally, there is a question about the section one trees. Section one has the most level topography, affecting infiltration. This may indicate that geographical location within the ERCO plantation of each section is an additional source of variation.

Keeping these observations in mind, there are two general trends that emerge. First, the age 1 trees tend to have lower foliar concentrations of most nutrients due to a root system that is small and still developing. Second, many foliar nutrient

concentrations peak between the ages of 3 and 5. This is indicative of inadequate nutrient availability and is also in agreement with previous observations at the ERCO. This is most likely a result of the depletion of nutrients in the biosolids by the trees.

Modeling

In general, the models constructed from the foliar and spectral data were inadequate predicators of foliar nutrient concentrations. While the full cross-validation models showed promise (slope of the Predicted vs. Measured regression line greater than 0.50 and r-squared values greater than 0.50) in quite a few instances, the test set models did not bear out this promise. However, the use of a random test set did improve the performance of the models for N, Cu, and Al and this method should be evaluated further. The year to year variations of most of the data combined with the impact of differing yearly levels of precipitation likely play a role in the failure of the models to adequately predict foliar concentrations from spectral data. Six nutrients, H, N, P, K, Cu and Al, did show promise (slope of the Predicted vs. Measured regression line greater than 0.50 and r-squared values greater than 0.50 in at least part of one model) to warrant further study. While H is a relatively uninteresting nutrient, the other nutrients are of interest, both financially and regulatory, to growers of hybrid poplar and to those who utilize biosolids as a crop fertilizer source.

Leaf Water Potential

Leaf water potential measurements were only taken in one year, 2004. Collection problems presented in the following years, and the solutions proposed by

PMS Industries were not practical or possible under field conditions. The measurements were also exacerbated by the environmental conditions at the ERCO site. With the exception of a few high intensity short duration storms, the 2005 growing season was quite dry. This resulted in inordinately high readings from the pressure bomb. The solution proposed was to stop leaf transpiration by placing the leaves that were to be sampled in foil lined bags for 40 minutes. However, this was not possible due to the height of the trees.

Future Work

The collection and analysis of the data generated by this study has resulted in some avenues for research that should be considered. First, it may be useful to grow the hybrid poplar under controlled conditions where nutrient concentrations and the amount of water available are easily controlled. Under these conditions it would be much easier to determine the spectral characteristics of each nutrient deficiency. Second, it would be interesting to see how the trees at the ERCO plantation respond to a foliar chelated iron spray. Third, an evaluation of the steckings would be interesting. There is variation in the diameter of the steckings and it would be interesting to see if this translates in different growth parameters, either initially or after the first few years of growth. Next, a time course study of spectral reflectance and foliar nutrient concentrations would be interesting in order to determine optimal sampling times for other nutrients. Finally, it would be interesting to see if an index could be developed using lateral leaves and tree height in predicting leaf water potential. Lateral leaves are much easier to collect than leaves growing in the upper crown.

Appendix 1 – Unremarkable Foliar Data

2004

Carbon

The results of the foliar carbon testing are found in Table 87. The samples tested in 2004 were transformed using log and square root functions. None of the transformations improved the graphs of the residuals or the value of the Shapiro-Wilk test statistic when compared to the values generated from untransformed data. Both the raw data and the transformed data sets had a Levene's HOV test p-value greater than 0.05, indicative of equal variances among the means. Thus, the untransformed data were used in the ANOVA. The ANOVA results using a Tukey adjustment are presented in Table 87. These results indicate that there is no significant difference between the different ages with respect to foliar carbon concentrations.

Table 87. 2004 ANOVA for Foliar Carbon (%)

Age	Mean	SD	N	Tukey
1	48.24	0.74	10	A
2	47.94	0.43	10	A
3	48.11	0.77	10	A
4	47.94	0.56	10	A
5	47.96	0.51	10	A

Means with the same letter are not significantly different

Sulfur

Foliar testing results for sulfur are found in Table 88. Based on the evaluation of the performance metrics, the foliar sulfur data for 2004 did not require any transformations applied. The results of an ANOVA with Tukey adjustment on the untransformed data are found in Table 88. The results indicate that there were no

significant differences in foliar sulfur levels between the different ages of trees for the year 2004.

Table 88. 2004 ANOVA for Foliar Sulfur (%)

Age	Mean	SD	N	Tukey
1	0.30	0.04	10	A
2	0.34	0.05	10	A
3	0.34	0.03	10	A
4	0.32	0.04	10	A
5	0.35	0.06	10	A

Means with the same letter are not significantly different

Copper

Copper foliar testing results are found in Table 89. While the graphs of the residuals did not indicate that data transformations were necessary, log transforming the data improved the value of the Shapiro-Wilk statistic from 0.8746 to 0.9561. In addition, log transformation significantly improved kurtosis from 6.2351 to 1.6088. The ANOVA results on the log transformed data using a Tukey adjustment are presented in Table 89. These results indicate that in 2004 there were no significant differences in foliar copper levels between the different ages of trees.

Table 89. 2004 ANOVA for Foliar Copper (ppm)

Age	Mean	SD	N	Tukey
1	7.31	1.22	10	A
2	7.86	2.46	10	A
3	6.15	1.06	10	A
4	6.86	1.66	10	A
5	6.51	0.60	10	A

Means with the same letter are not significantly different

2005

Phosphorus

Foliar phosphorus results are found in Table 90. Log transformation of the 2005 foliar phosphorus data decreased the amount of skew and kurtosis while

increasing the value of the Shapiro-Wilk test statistic. The results of an ANOVA with Tukey adjustment on the log transformed data are found in Table 90. The results indicate that there were no significant differences in foliar phosphorus concentrations between the different ages of trees during 2005. Additionally, all of the different ages of trees have mean values for foliar phosphorus that are less than the ideal level of 0.30% needed for rapid growth.

Table 90. 2005 ANOVA for Foliar Phosphorus (%)

Age	Mean	SD	N	Tukey
2	0.22	0.05	4	A
3	0.20	0.03	4	A
4	0.21	0.02	4	A
5	0.20	0.02	4	A

Means with the same letter are not significantly different

Potassium

The results for foliar potassium are found in Table 91. Transformations of the 2005 foliar potassium data resulted in marginal, if any, improvement. Thus, the untransformed data were used to generate an ANOVA. The results of the ANOVA with Tukey adjustment, found in Table 91, show that there were no significant differences in foliar potassium concentrations between the different ages of trees for the 2005 data.

Table 91. 2005 ANOVA for Foliar Potassium (%)

Age	Mean	SD	N	Tukey
2	1.48	0.25	4	A
3	1.58	0.12	4	A
4	1.44	0.08	4	A
5	1.53	0.05	4	A

Means with the same letter are not significantly different

Sulfur

The results for foliar sulfur are found in Table 92. The foliar sulfur data for 2005 did not need transformation based on the different performance metrics. However, the p-value of 0.0106 for Levene's HOV test on untransformed data indicated that there were unequal variances. No transformations improved the p-value of Levene's HOV test. Thus, a Welch's test was performed on the untransformed data to test the equality of the means for the different ages. The p-value of 0.1101 for the Welch's test showed that there were homogeneous age means. Thus, the untransformed data were used to generate an ANOVA. The results of an ANOVA with Tukey adjustment are found in Table 92. The results indicate that similar to 2004, there were no significant differences in foliar sulfur levels between the different ages of trees for the year 2005.

Table 92. 2005 ANOVA for Foliar Sulfur (%)

Age	Mean	SD	N	Tukey
2	0.42	0.08	4	A
3	0.43	0.03	4	A
4	0.52	0.06	4	A
5	0.49	0.03	4	A

Means with the same letter are not significantly different

Iron

Foliar iron results are found in Table 93. The evaluation of the metrics for the 2005 data indicate that the transformed data did not appreciable improves any results. Thus, the untransformed data were used for the ANOVA. The results of the ANOVA with Tukey adjustment are found in Table 93. The results indicate that for 2005 there were no significant differences between the different ages of trees with respect to foliar iron concentrations.

Table 93. 2005 ANOVA for Foliar Iron (ppm)

Age	Mean	SD	N	Tukey
2	178.25	36.12	4	A
3	159.75	30.51	4	A
4	163.75	37.38	4	A
5	130.75	7.18	4	A

Means with the same letter are not significantly different

Copper

Foliar copper results are found in Table 94. Similar to 2004, the graphs of the residuals for the 2005 data did not indicate that a data transformation was necessary. However, log transforming the data improved the value of the Shapiro-Wilk statistic from 0.8568 for untransformed data to 0.9311 for log transformed data and decreased kurtosis from 2.7710 for untransformed data to 0.8746 for log transformed data. Thus, log transformed data were used in the ANOVA. The results of the ANOVA with a Tukey adjustment are presented in Table 94. Like 2004, the results for 2005 indicate that there was no significant difference between the different ages of trees with respect to foliar copper concentrations.

Table 94. 2005 ANOVA for Foliar Copper (ppm)

Age	Mean	SD	N	Tukey
2	13.25	1.71	4	A
3	15.25	1.71	4	A
4	17.00	4.00	4	A
5	13.25	1.26	4	A

Means with the same letter are not significantly different

Manganese

Foliar manganese results are found in Table 95. The graph of the residuals for the 2005 foliar manganese data suggested that a log transformation would improve the distribution of the data. The log transformation also had the best improvement for the value of the Shapiro-Wilk test statistic, from 0.08065 for untransformed data to

0.8846 for log transformed data, of any transformation used. The ANOVA results with a Tukey adjustment on the log transformed foliar manganese data are presented in Table 95. The results indicate that there were no significant differences in foliar manganese levels between the different ages of trees for the year 2005.

Table 95. 2005 ANOVA for Foliar Manganese (ppm)

Age	Mean	SD	N	Tukey
2	223.50	52.22	4	A
3	307.00	118.11	4	A
4	345.25	305.25	4	A
5	243.25	13.72	4	A

Means with the same letter are not significantly different

Zinc

Results for foliar zinc are found in Table 96. Transformation of 2005 data for foliar zinc was not necessary based on the graphs of the residuals and the p-value of Levene's HOV test. The results of an ANOVA with a Tukey adjustment on the untransformed data are presented in Table 96. The results indicate that for 2005 there were no significant differences between the different ages of trees with respect to foliar zinc concentrations.

Table 96. 2005 ANOVA for Foliar Zinc (ppm)

Age	Mean	SD	N	Tukey
2	75.50	14.39	4	A
3	83.00	10.89	4	A
4	63.75	20.34	4	A
5	93.25	10.84	4	A

Means with the same letter are not significantly different

Sodium

Foliar sodium results for 2005 are found in Table 97. Statistical analysis of the 2005 data was not performed due to the fact that identical values for foliar sodium

concentrations were reported for all samples. Thus, there is no difference in foliar sodium levels between the different ages of trees for the year 2005.

Table 97. 2005 Foliar Sodium Concentrations (%)

Age	Mean	SD	N
2	0.01	0	4
3	0.01	0	3
4	0.01	0	4
5	0.01	0	4

Aluminum

The results for the 2005 foliar aluminum data are found in Table 98. The graph of the residuals suggested that the square root transformation was necessary. The Levene's HOV test and the value of the Shapiro-Wilk test statistic of the square root transformed data were both indicative of equal variances within each age and of adequate normality. Thus, the square root transformed data were used in the ANOVA, and the ANOVA results using a Tukey adjustment are presented in Table 98. The general trend for the 2005 foliar aluminum data indicates a plateau for all ages. These results indicate that there is no significant difference between the different ages of trees with respect to foliar aluminum concentrations for the year 2005

Table 98. 2005 ANOVA for Foliar Aluminum (ppm)

Age	Mean	SD	N	Tukey
2	68.50	16.84	4	A
3	77.50	24.69	4	A
4	96.75	38.28	4	A
5	61.75	11.76	4	A

Means with the same letter are not significantly different

2006

Zinc

Foliar zinc results for 2006 are found in Table 99. The data were log transformed based on the improvement in the graphs of the residuals when compared to untransformed data. The results of an ANOVA with a Tukey adjustment on the log transformed foliar data are presented in Table 99. The results indicate that there were no significant differences in foliar zinc concentrations between the different ages of trees during the year 2006.

Table 99. 2006 ANOVA for Foliar Zinc (ppm)

Age	Mean	SD	N	Tukey
0	58.20	17.06	5	A
1	53.40	22.90	5	A
2	66.00	19.86	5	A
3	55.60	12.22	5	A
4	52.40	10.29	5	A
5	53.60	10.43	5	A
6	62.60	13.11	5	A
7	48.40	2.70	5	A

Means with the same letter are not significantly different

2007

Potassium

Foliar potassium results for 2007 are found in Table 100. Log transformation of the data improved the values for all of the performance metrics. However, the p-value of 0.0156 for Levene's HOV test on the log transformed data indicated that the variances within the ages were not the same. A Welch's test was performed to test the equality of the means for the different ages. The p-value of 0.1214 for the Welch's test showed that there were homogeneous age means. Thus, the log

transformed data were used to generate an ANOVA. The results of an ANOVA with Tukey adjustment on the log transformed data, found in Table 100, show that there were no significant differences in foliar potassium concentrations between the different ages of trees for the year 2007. The lower levels of foliar potassium are likely a reflection of the amount of rainfall at the ERCO tree farm. In 2007, the months of June and July saw a combined total of 3.61 inches of rainfall. Potassium is very mobile in water and is easily taken up and transported between plant tissues. In all likelihood, the limited amount of rainfall likely had a negative effect on potassium uptake and transport by limiting potassium movement in the soil through the root zone, resulting in markedly lower foliar potassium levels than the observed in previous years.

Table 100. 2007 ANOVA for Foliar Potassium (%)

Age	Mean	SD	N	Tukey
2	1.03	0.22	5	A
4	0.84	0.12	5	A
5	0.91	0.10	5	A
6	0.79	0.12	5	A
7	0.96	0.05	4	A

Means with the same letter are not significantly different

Copper

The results for foliar copper are found in Table 101. Log transformation of the data was used based on the decrease in the amount of skew evident in the boxplot of the residuals. While log transforming the data decreased the p-value of Levene's HOV test from 0.1530 to 0.0522 when compared with untransformed data, the transformed p-value is still evident of equal variances. The results of an ANOVA with Tukey adjustment on log transformed data are presented in Table 101. The data

indicates that in 2007 there were no significant differences in foliar copper levels for any of the different ages of trees.

Table 101. 2007 ANOVA for Foliar Copper (ppm)

Age	Mean	SD	N	Tukey
2	9.60	1.52	5	A
4	9.00	0.00	5	A
5	9.60	1.52	5	A
6	8.00	0.71	5	A
7	8.50	1.73	4	A

Means with the same letter are not significantly different

Zinc

Results for foliar zinc are found in Table 102. Log transforming the foliar zinc data for 2007 improved the distribution in the graph of the residuals while having no overall negative impact on the values for the Shapiro-Wilk test statistic or the p-value for Levene's HOV test. Thus, log transformed data were used to generate an ANOVA with a Tukey adjustment. The results of the ANOVA are presented in Table 102. These results indicate that similar to 2005 and 2006, there were no significant differences in foliar zinc levels between the different ages of trees for 2007.

Table 102. 2007 ANOVA for Foliar Zinc

Age	Mean	SD	N	Tukey
2	64.00	25.14	5	A
4	41.20	13.48	5	A
5	84.20	37.53	5	A
6	80.80	28.96	5	A
7	53.25	11.56	4	A

Means with the same letter are not significantly different

Sodium

Foliar sodium results for 2007 are found in Table 103. Data transformations did not result in any improvements in the performance metrics. Thus, untransformed data were used in the ANOVA, and the results of the ANOVA using a Tukey

adjustment are presented in Table 103. There were no significant differences between the different ages of trees with respect to foliar sodium levels for 2007.

Table 103. 2007 ANOVA for Foliar Sodium (%)

Age	Mean	SD	N	Tukey
2	0.016	0.005	5	A
4	0.010	0.000	5	A
5	0.014	0.005	5	A
6	0.012	0.004	5	A
7	0.018	0.004	4	A

Means with the same letter are not significantly different

Appendix 2 – Rainfall Data

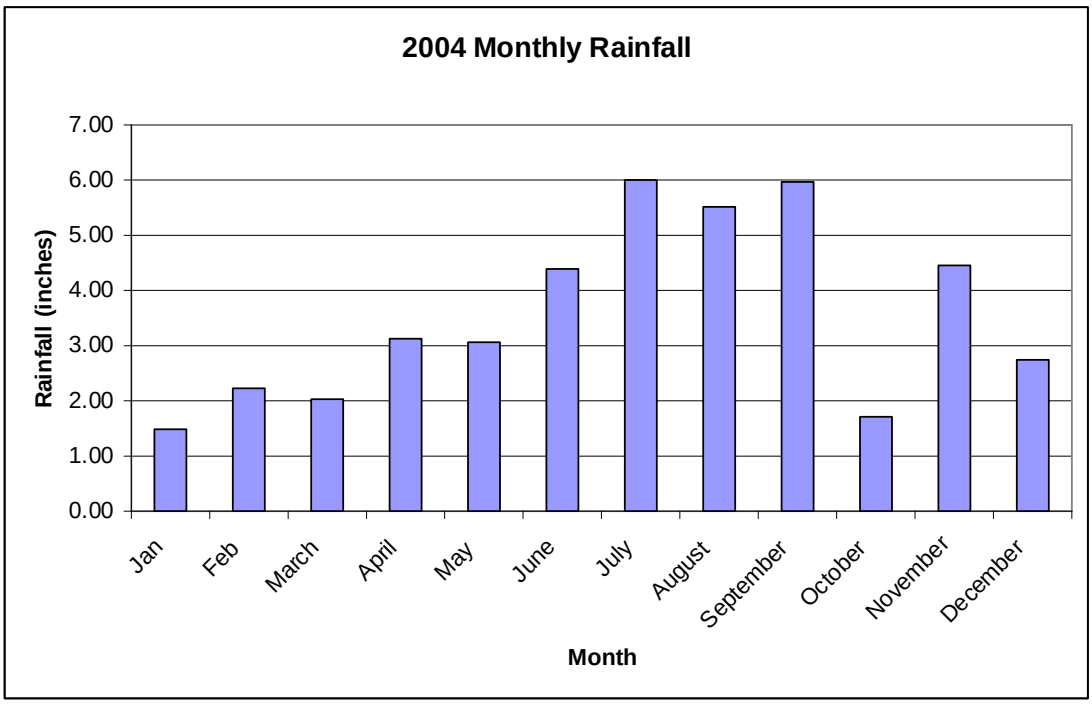

Figure 21. 2004 Monthly Rainfall

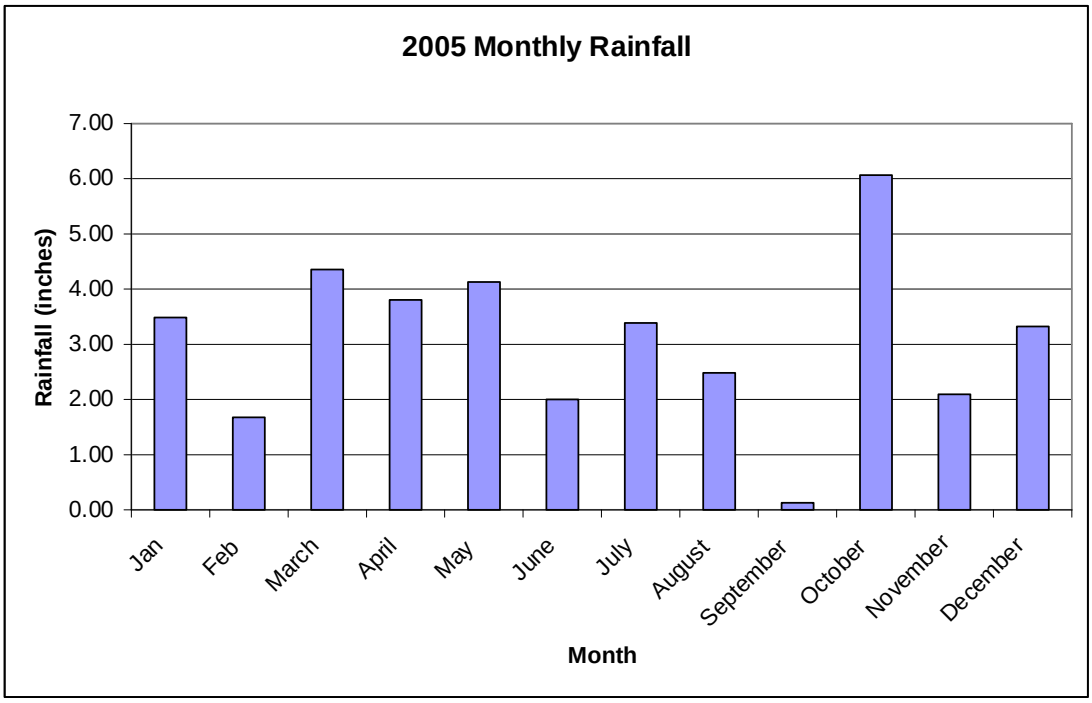

Figure 22. 2005 Monthly Rainfall

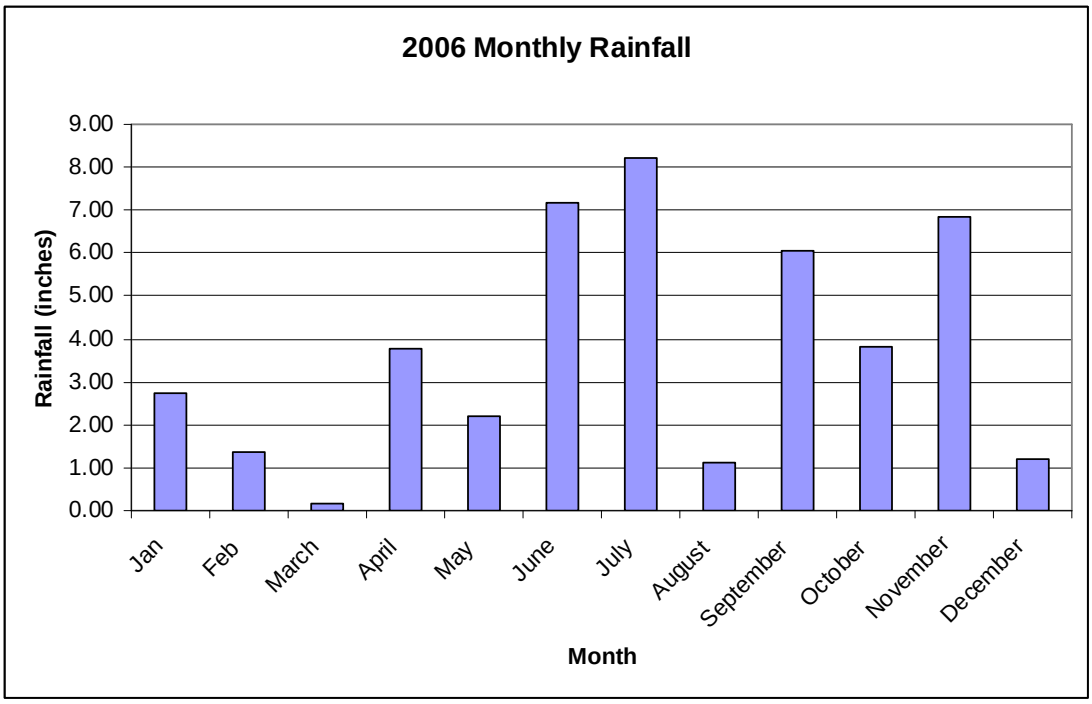

Figure 23. 2006 Monthly Rainfall

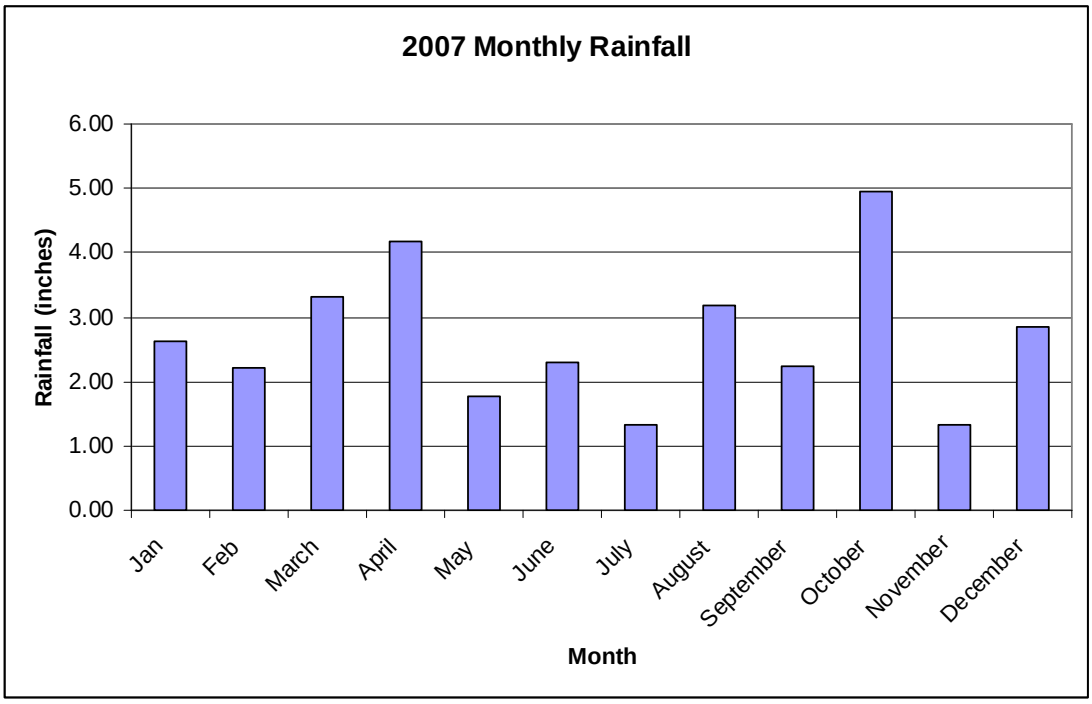

Figure 24. 2007 Monthly Rainfall

Appendix 3 – Foliar Data Summary

Table 104. 2004 Foliar Data Summary

Year	2004	2004	2004	2004	2004
age	1	2	3	4	5
n	10	10	10	10	10
bar	25.850	20.75	25.05	25.75	21
weight	0.137	0.149	0.150	0.151	0.148
%C	48.243	47.936	48.108	47.944	47.960
%H	5.040	5.183	5.186	5.644	5.617
% N	2.577	3.083	3.076	3.218	3.281
% P	0.220	0.252	0.271	0.327	0.364
% K	1.226	1.328	1.584	2.231	1.701
% Ca	1.411	1.234	1.049	0.906	1.106
% Mg	0.406	0.364	0.327	0.256	0.312
% S	0.298	0.340	0.337	0.325	0.351
Mn ppm	177.7	228.1	160.0	73.8	52.3
Zn ppm	102.0	68.1	54.4	69.0	73.4
Cu ppm	7.3	7.9	6.2	6.9	6.5
Fe ppm	181.6	157.6	94.4	89.0	89.9
% Na	NA	NA	NA	NA	NA
B ppm	NA	NA	NA	NA	NA
Al ppm	NA	NA	NA	NA	NA

Table 105. 2005 Foliar Data Summary

Year	2005	2005	2005	2005
age	2	3	4	5
n	4	4	4	4
bar	NA	NA	NA	NA
weight	NA	NA	NA	NA
%C	47.223	47.175	46.733	47.438
%H	NA	NA	NA	NA
% N	3.225	3.295	3.165	2.868
% P	0.220	0.200	0.213	0.198
% K	1.478	1.583	1.438	1.533
% Ca	1.000	1.153	1.738	1.353
% Mg	0.383	0.345	0.325	0.268
% S	0.423	0.430	0.518	0.485
Mn ppm	223.5	307.0	345.3	243.3
Zn ppm	75.5	83.0	63.8	93.3
Cu ppm	13.3	15.3	17.0	13.3
Fe ppm	178.3	159.8	163.8	130.8
% Na	0.010	0.033	0.010	0.010
B ppm	34.5	30.0	50.3	62.3
Al ppm	68.5	77.5	96.8	61.8

Table 106. 2006 Foliar Data Summary

Year	2006	2006	2006	2006	2006	2006	2006	2006
age	0	1	2	3	4	5	6	7
n	5	5	5	5	5	5	5	5
bar	NA	NA	NA	NA	NA	NA	NA	NA
weight	NA	NA	NA	NA	NA	NA	NA	NA
%C	NA	NA	NA	NA	NA	NA	NA	NA
%H	NA	NA	NA	NA	NA	NA	NA	NA
% N	3.136	2.134	3.292	3.404	3.426	3.544	4.034	3.332
% P	0.364	0.280	0.362	0.288	0.284	0.418	0.470	0.352
% K	3.332	1.852	2.768	1.880	2.448	2.630	3.768	3.042
% Ca	1.072	1.066	1.018	0.802	1.216	0.894	1.060	0.924
% Mg	0.200	0.244	0.214	0.384	0.366	0.268	0.302	0.262
% S	0.386	0.242	0.330	0.322	0.352	0.338	0.406	0.326
Mn ppm	58.6	84.2	63.6	129.6	147.8	76.6	133.6	74.8
Zn ppm	58.2	53.4	66.0	55.6	52.4	53.6	62.6	48.4
Cu ppm	11.2	7.4	10.2	9.4	11.4	8.8	10.4	10.4
Fe ppm	90.0	109.4	81.8	125.4	89.6	85.0	76.0	95.0
% Na	0.018	0.012	0.014	0.010	0.014	0.010	0.010	0.012
B ppm	26.6	28.4	31.4	24.2	32.8	46.2	42.6	35.4
Al ppm	26.8	20.4	11.8	13.8	16.2	9.2	7.0	14.4

Table 107. 2007 Foliar Data Summary

Year	2007	2007	2007	2007	2007
age	2	4	5	6	7
N	5	5	5	5	5
bar	NA	NA	NA	NA	NA
weight	NA	NA	NA	NA	NA
%C	NA	NA	NA	NA	NA
%H	NA	NA	NA	NA	NA
% N	2.860	3.246	3.126	2.900	2.322
% P	0.184	0.136	0.146	0.172	0.134
% K	1.032	0.842	0.914	0.790	0.952
% Ca	1.928	1.084	1.716	2.156	2.488
% Mg	0.282	0.502	0.400	0.416	0.304
% S	0.376	0.274	0.344	0.392	0.452
Mn ppm	103.8	231.8	378.8	228.0	180.4
Zn ppm	64.0	41.2	84.2	80.8	52.4
Cu ppm	9.6	9.0	9.6	8.0	8.2
Fe ppm	111.6	96.8	94.6	78.8	100.0
% Na	0.016	0.010	0.014	0.012	0.018
B ppm	35.0	28.0	39.4	70.8	69.2
Al ppm	26.2	26.2	23.4	18.0	35.4

Appendix 4 – Spectral Data Summary

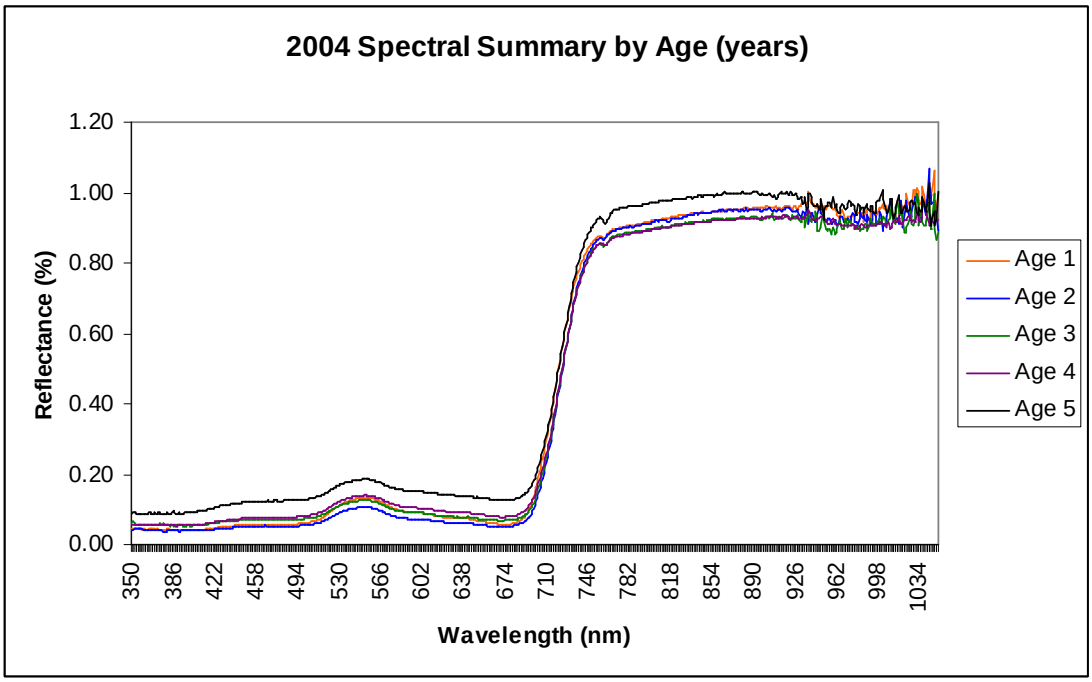

Figure 25. 2004 Spectral Summary

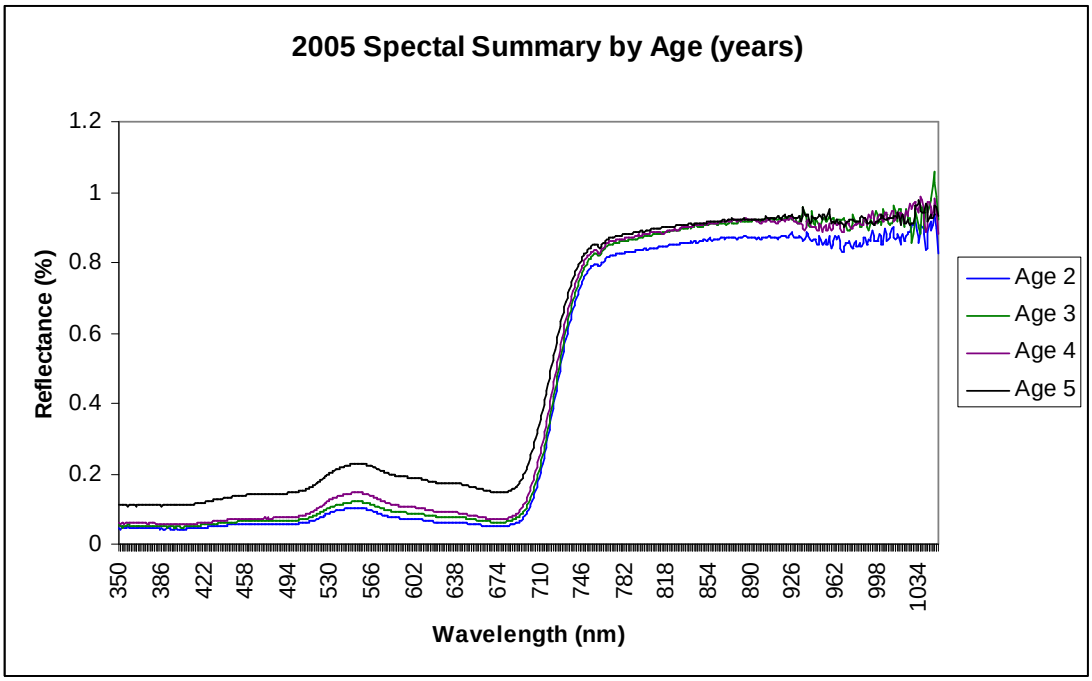

Figure 26. 2005 Spectral Summary

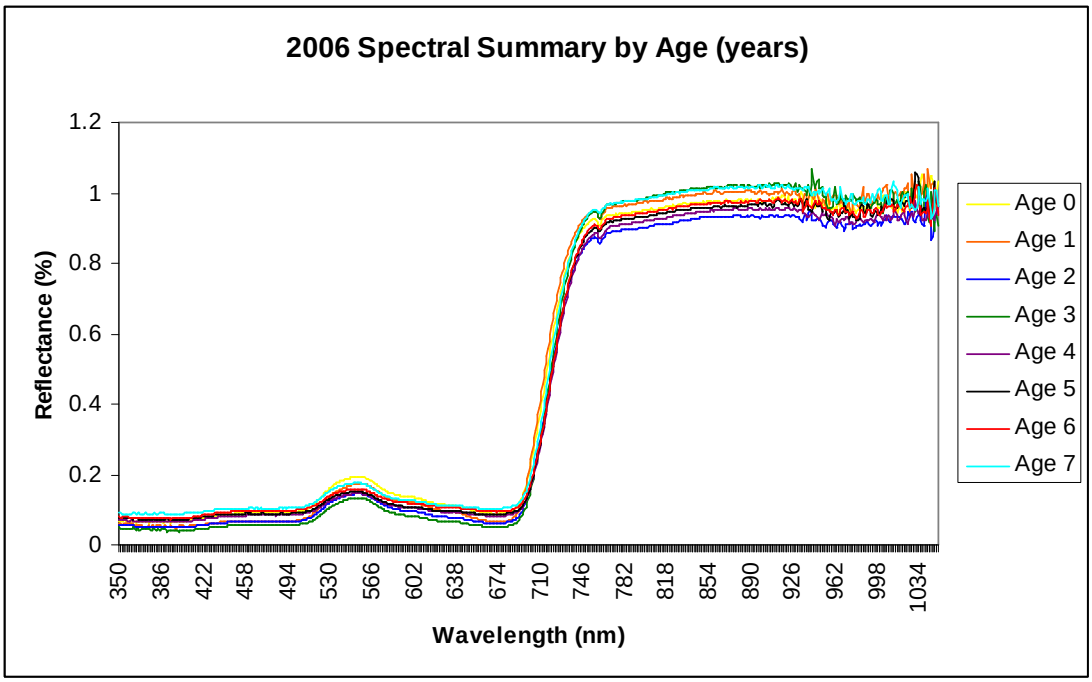

Figure 27. 2006 Spectral Summary

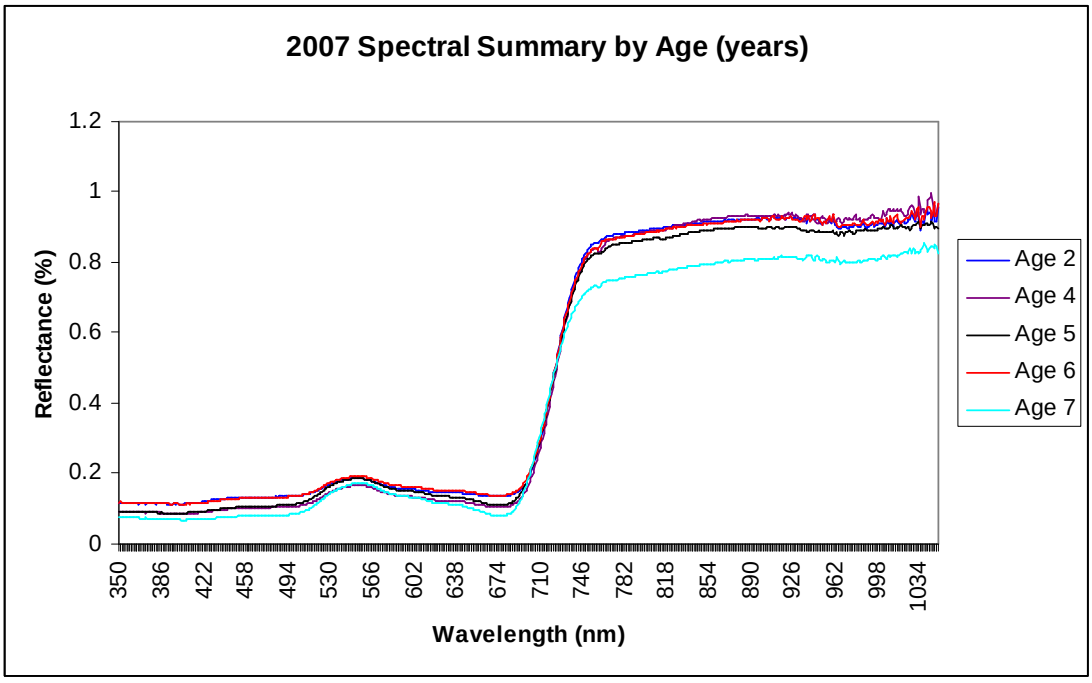

Figure 28. 2007 Spectral Summary

Appendix 5 – SAS Codes

The following is an example of the SAS code used to analyze nitrogen in 2004. The analysis for the years 2005-2007 used the same code but different source datasets corresponding to the year of interest. The analysis of other nutrients was accomplished by simply changing the name from nitrogen to the nutrient to be evaluated.

```
dm 'log;clear;out;clear;';
PROC IMPORT OUT= WORK.sasdata
            DATAFILE= "L:\Analysis\SAS\2004\2004 sas data.xls"
            DBMS=EXCEL2000 REPLACE;
     GETNAMES=YES;
RUN;
proc print data=sasdata;
run;
proc glm data=sasdata;
class age;
model N=age/ss3;
means age/hovtest;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
data newsasdataN;
set sasdata;
log_N=log10(N);
sq_N=sqrt(N);
run;
proc print data=newsasdataN;
run;
proc glm data=newsasdataN;
class age;
model log_N=age/ss3;
means age/hovtest;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
```

```
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
proc glm data=newsasdataN;
class age;
model sq_N=age/ss3;
means age/hovtest;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
proc mixed data=sasdata cl;
class age;
model N=age/ddfm=kr outp=resids;
lsmeans age/pdiff adjust=tukey;
run;
```

The following is an example of the SAS code used to analyze nitrogen for 2004-2007. The analysis of other nutrients was accomplished by simply changing the name from nitrogen to the nutrient to be evaluated.

```
dm 'log;clear;out;clear;';
PROC IMPORT OUT= WORK.sasdata
            DATAFILE= "L:\Analysis\SAS\Combined Nutrient Data 2004-
2007\2004-2007 combined nutrient data.xls"
            DBMS=EXCEL2000 REPLACE;
     GETNAMES=YES;
RUN;
proc print data=sasdata;
run;
proc glm data=sasdata;
class age;
model N=age/ss3;
means age/hovtest=levene welch;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
```

```
run;
data newsasdataN;
set sasdata;
log_N=log10(N);
sq_N=sqrt(N);
*arc_N=arsin(N)*57.2958;
run;
proc print data=newsasdataN;
run;
proc glm data=newsasdataN;
class age;
model log_N=age/ss3;
means age/hovtest=levene welch;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
proc glm data=newsasdataN;
class age;
model sq_N=age/ss3;
means age/hovtest=levene welch;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
proc glm data=newsasdataN;
class age;
model arc_N=age/ss3;
means age/hovtest=levene welch;
means age/tukey lines;
output OUT=resids P=pred R=resid;
run;
proc print data=resids;
run;
proc univariate data=resids normal plot;
var resid;
run;
proc plot data=resids;
plot resid*pred/vref=0;
plot resid*age/vref=0;
run;
```

```
proc mixed data=sasdata cl;
class age;
model N=age/ddfm=kr outp=resids;
lsmeans age/pdiff adjust=tukey;
run;
```

Appendix 6 – PLS Graphs

This appendix contains an example of the PLS graphs used for the interpretation of the nitrogen model. Similar graphs for other nutrients were used in the interpretation of the model that particular nutrient.

Nitrogen

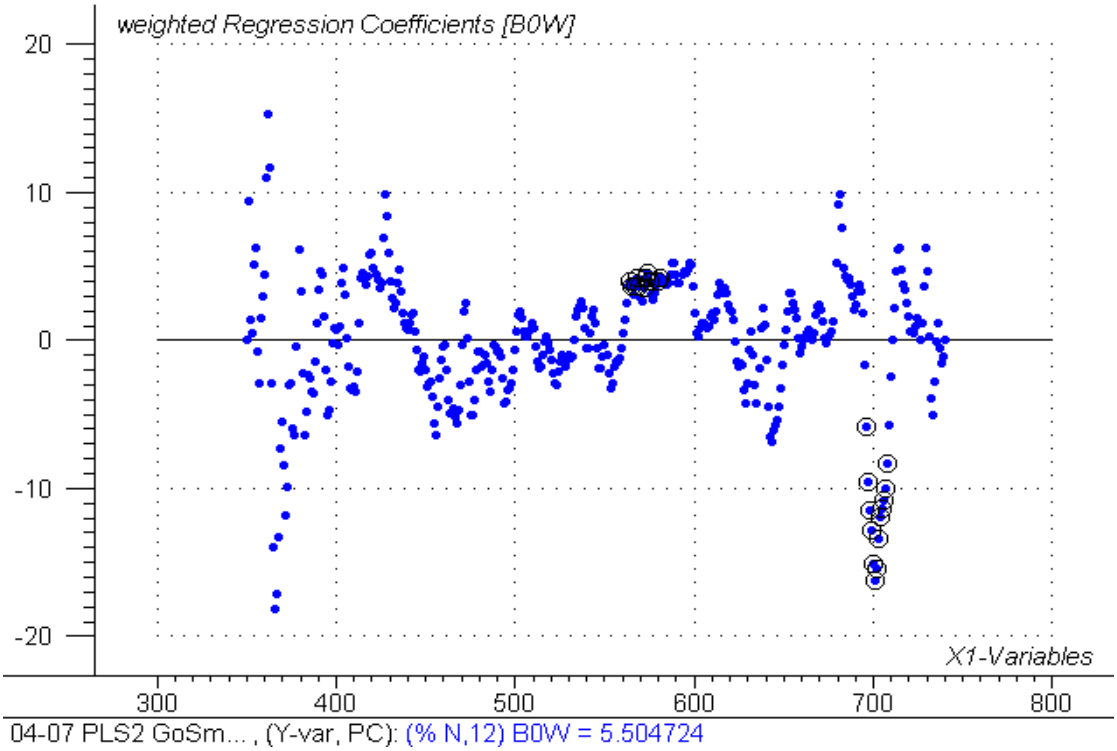

Figure 29. Regression Coefficients for Nitrogen from Full Cross-validation Model

Figure 29 shows the graph of the regression coefficients for the full cross-validation nitrogen model. The black circles indicate significant wavebands in the model. In this case the significant wavebands correspond to the regions influenced by the chlorophyll molecule.

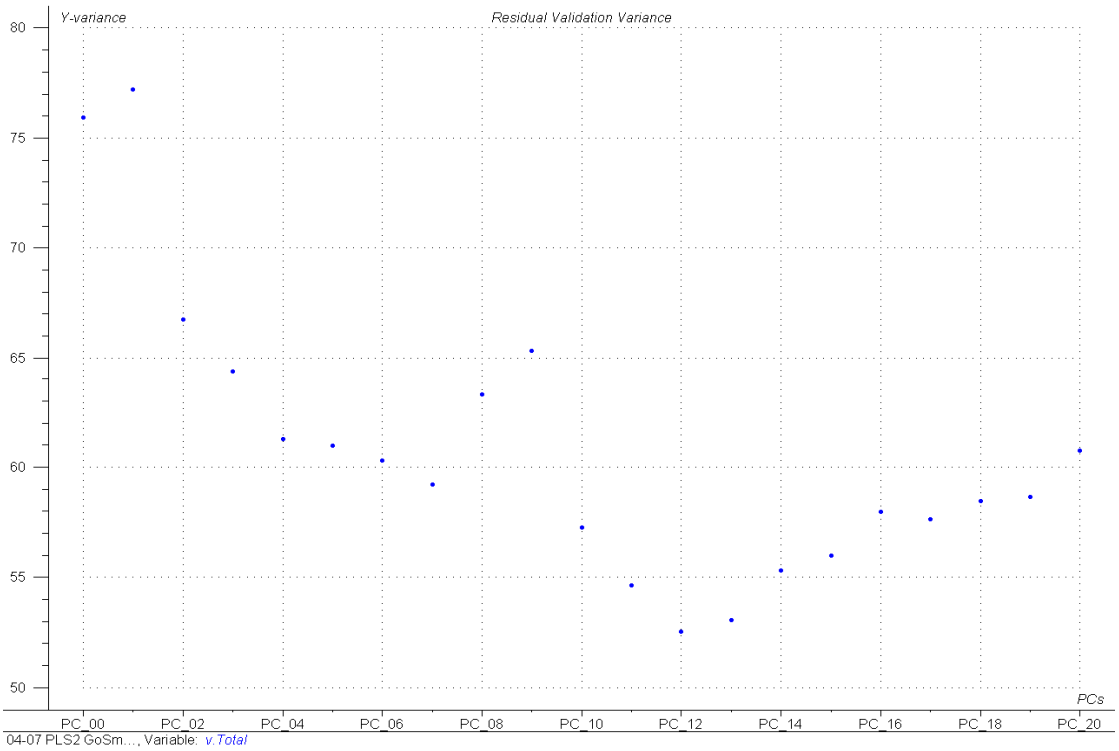

Figure 30. Global Variance from Full Cross-Validation Model for Nitrogen

Figure 30 shows the global variance for the full cross-validation model. This figure indicates a global minimum at 12 PCs. The number of PCs used in a model is based on the number of PCs with the lowest global variance. Thus, 12 PCs were used when interpreting this model.

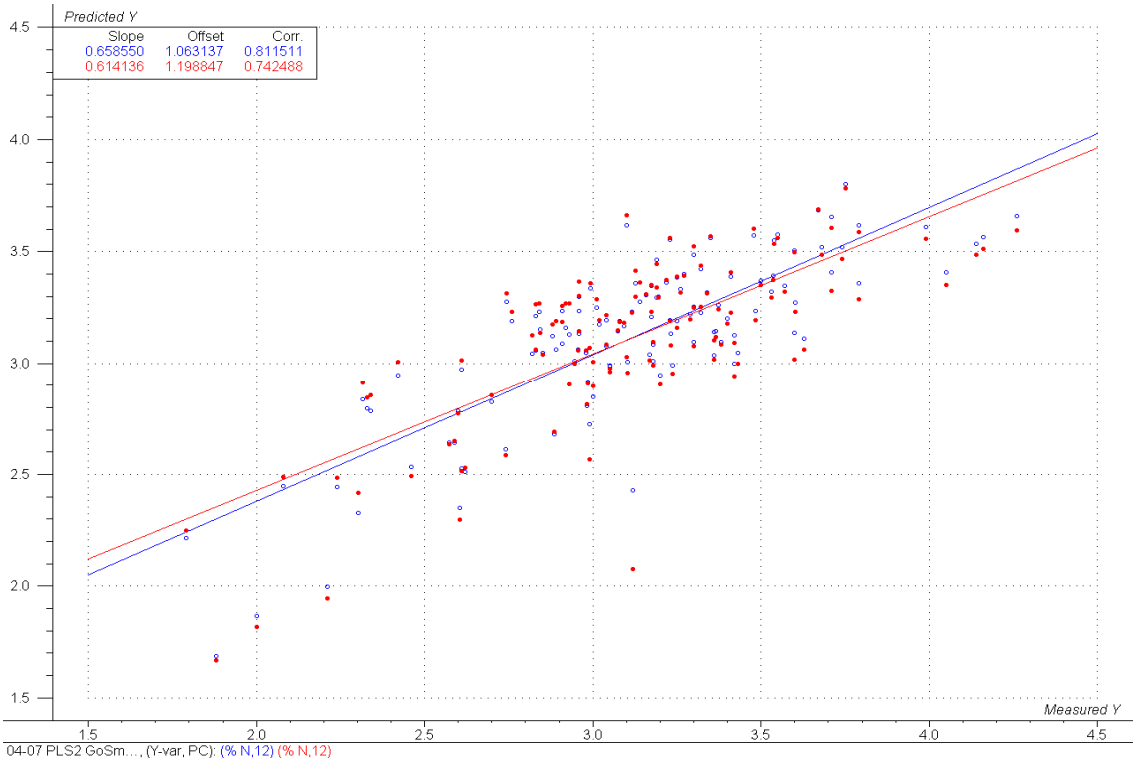

Figure 31. Predicted vs. Measured foliar Nitrogen concentrations from Full Cross-Validation Model

Figure 31 shows the graph of the Predicted vs. Measured for foliar nitrogen from the full cross-validation model based on 12 PCs. The calibration portion of the model is in blue and the validation portion is in red. The slope of the calibration and validation regression lines, both greater than 0.50, and the correlation (converted into r-squared) both indicate that the full cross-validation model does appear to predict foliar nitrogen concentrations from reflectance data.

Figure 32. Predicted vs. Measured foliar Nitrogen concentrations from Test Set Model

Figure 32 shows the Predicted vs. Measured results for foliar nitrogen from the test set model using 2004-2006 data for calibration and 2007 data for validation. slope of the calibration and validation regression lines, both less than 0.50, and the correlation (converted into r-squared) of the model indicate that the test set model does not adequately predict foliar nitrogen concentrations from reflectance data.

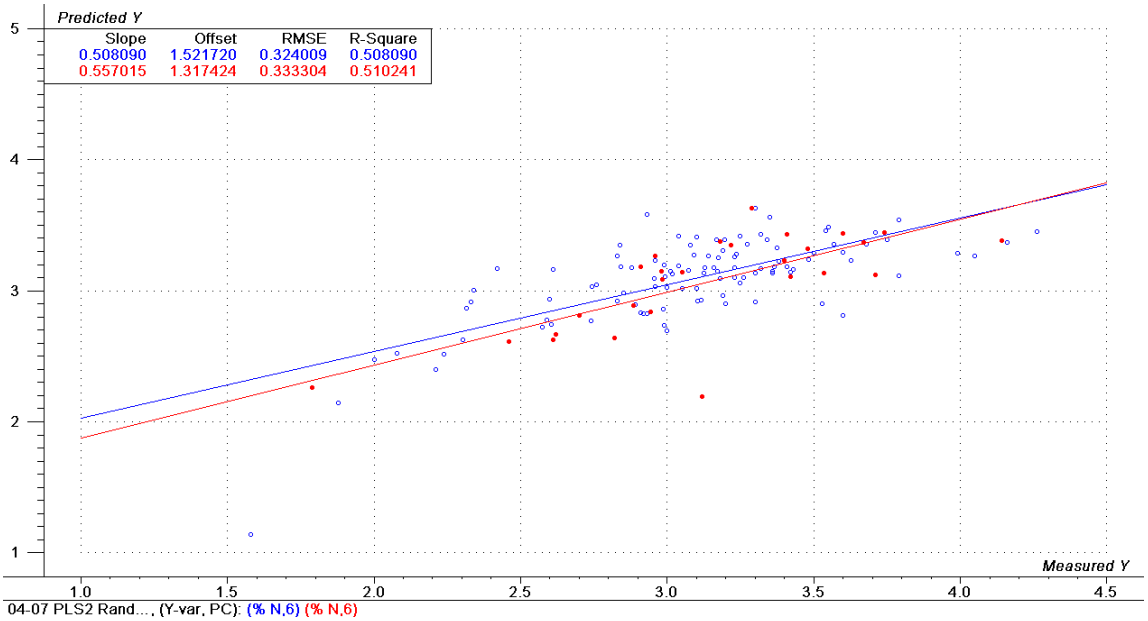

Figure 33. Predicted vs. Measured foliar Nitrogen concentrations from Test Set Model using Random Test Set

Figure 33 shows the Predicted vs. Measured results for foliar nitrogen from the test set model using the 2004-2007 data for calibration and a validation set that contained a random selection of 20% of the samples from each age in each year. For example, two of the 10 trees sampled from each age of tree, ages 1 through 5, in 2004 were randomly selected and set aside as part of the test set for a total of 10 trees in the test set from 2004. This was repeated for each age in every year for a total of 27 samples in the test set. The slope of the regression lines and the values of r-squared for both portions of the model, shown in Figure 33, indicates that the test set model utilizing a random selection of samples adequate models foliar nitrogen concentrations from reflectance data. The differences between model prediction shown in Figures 32 and 33 indicate the importance of sample selection in the generation of the test set.

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