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# Effects of Excessive Soil Phosphorus Accumulation on Loblolly Pine (Pinus taeda L.) Seedlings

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# Effects of Excessive Soil Phosphorus Accumulation on Loblolly Pine (*Pinus taeda* L.) Seedlings

By

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## ABSTRACT

Many landowners in East Texas apply poultry litter to pastures as a source of nitrogen (N) for forage crops. After many years of repeated poultry litter application, soils can accumulate extremely high extractable phosphorus (P) concentrations, sometimes over 1000 mg $kg^{-1}$ of plant available P. Landowners report the conversion of these pastures to loblolly pine (*Pinus taeda* L.) plantations is often problematic, with poor seedling survival and abnormal tree growth. This study was conducted to assess the effects of excessive soil phosphorus on loblolly pine seedlings. An outdoor pot study was conducted using bare root seedlings and triple superphosphate treatments to simulate different soil P concentrations of those from poultry litter applications. The treatments were a control, 250 mg $kg^{-1}$, 500 mg $kg^{-1}$, 750 mg $kg^{-1}$, 1000 mg $kg^{-1}$, and 1250 mg $kg^{-1}$of plant available P with eight replications. Seedlings were grown for one growing season and measured periodically for survival, height and diameter growth, foliage color using a Munsell color transformation, and at the end of the study sampled for dry biomass (above and below ground), survivability, foliar nutrient content, and ground needle color Munsell color transformation comparison. Growth trends in the study were positive for growth relationship to P treatment level increase. However, at the end of the first growing season seedlings presented deficiency symptoms like needle tissue chlorosis and branch tip necrosis in the high P treatments. Trends in foliar nutrient content were an

increase in zinc concentrations and a decrease in iron concentrations as phosphorus treatment level increased. Excess foliar phosphorus ratioed to iron and zinc emphasized a dilution effect or possible phosphorus:iron competitive interaction, especially with iron. Munsell color comparison between living and ground color showed a higher variability in ground color range in the 3-dimensional color space, as well as a higher significance between color and deficiency symptoms. Munsell color proved to be a useful tool to analyze the relationship between color and variable plant health.

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# INTRODUCTION

Demand for animal protein has risen to meet a rising global population (Blaizer et al. 2008). This has called for concentrated animal feeding operations (CAFOs) to increase productivity to meet the rising demand (Hribar 2010). In past decades, livestock farming has seen a significant transformation by decreasing the amount of time it takes an animal to reach market weight. The United States is a leading poultry producer and the second highest exporter of poultry meat (Grossen 2021). Within the United States 44.73 billion lbs. of poultry were produced in 2021, with East Texas producing roughly 10% of the total product (Southard 2004).

East Texas has seen its largest increase in timberland since 1975 with a 5% increase from what was reported in the 1992 inventory as of 2003 (Rudis et al. 2008). This increase can be attributed to landowners changing land use from pastureland, agricultural areas, and nonforest land, to pine plantations following financial incentives. These incentives include forestry and stewardship programs, the Forest Land Enhancement Program (2002), and the Texas Reforestation Foundation (1982). Recently, changes in property tax laws have allowed agricultural land converted to pine plantations to continue with a lower agricultural property tax rate, leading to an increase in conversion. Historically, much of the

agricultural and pastureland in East Texas has been fertilized with poultry litter due to regional availability, low monetary value, and high transportation costs. Subsequently, conversion of this land to loblolly pine plantations has sometimes been unsuccessful due to difficulty establishing and growing loblolly pine on soils with high available soil P content from past poultry litter fertilization. It should also be noted that agricultural and horticultural areas with high soil phosphorus experience decreased zinc and iron uptake (Cakmak & Marschner 1986, Drissi et al. 2015, Ova et al. 2015, Novais et al. 2016, Zhang et al. 2017)).

Poultry litter is a combination of both poultry manure, bedding material, feathers, and spilled feed. It contains all 13 essential nutrients that are necessary for all plant metabolic processes. These nutrients include: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), copper (Cu), zinc (Zn), chlorine (Cl), boron (B), iron (Fe), and molybdenum (Mo) (Ashworth et al. 2020). These nutrients are derived from supplements, feed, and water consumed by the animal. Poultry litter is mainly sourced as fertilizer for its N content, although only about 25 to 50% of organic N is readily available to plants within the first year of application depending on the prevailing environmental conditions (Zhang et al. 2013). The remaining nutrient content is mineralized through microbial activity, but is variable in most cases due to N not reaching full mineralization (Leikam & Lamond 2003). Other forms of release also consist of the gaseous release of N as ammonia ($NH_3$), which volatilizes into the atmosphere

depending on the chemical composition of available organic sources and soil processes before it can be utilized by the plants (Johnson et al. 2005, Rogers et al. 2018). Soil N varies greatly, but other macronutrients within poultry litter, such as P, are more stable within the environment due to the chemical bonds a phosphate ion can form. In most cases, poultry litter is used as a fertilizer at a rate that meets crop N requirements and will be the only fertilizer applied (Oldham 2021). Repeated poultry litter applications eventually produce high available soil P concentrations, sometimes greater than 1000 $mg\ kg^{-1}$. The litter is taken from poultry litter operations and transported to nearby agricultural fields, usually pastures for beef cattle, to increase forage productivity (Kulesza 2020). It is commonly used as a substitute for inorganic fertilizers, due to its desirable N content and its low cost.

There have been studies conducted to determine the nutritional content of poultry litter (Stephenson et al. 1990, Mitchell & Donald 1995, Ashworth et al. 2020), specifically how poultry litter should be applied and distributed (Wells & Allen 1985, Gaston et al. 2003, Liechty et al. 2009), and how vegetation responds to variable poultry litter concentrations (Sharpley et al. 1993, Warren & Fonteno 1993, Schutz 1997, Sistani et al. 2004, Colbert et al. 1990, Oldham 2021). General nutrient availability from applied nutrients and fertilizer is generally understood to potentially vary by region, climate, soil, and present vegetation.

The majority of poultry litter research is focused on water quality, but a fully comprehensive analysis would also incorporate the evaluation of crop development and soil health (Hoover et al. 2019). Due to excessive and long-term application of poultry litter over decades, nutrients like P have accumulated, whereas organic N has been depleted (Brink et al. 2002). In the past couple of decades, many landowners in the region have shifted from pasture and agricultural operations to timber production. Long term application in these areas has been reported to produce increased P, Zn, and Cu, which are contained in poultry litter (Foust et al. 2018, Kulesza & Sharara 2020). Cakmak & Marschner (1986) promotes that P-induced Zn deficiencies are not caused by an inhibition of Zn uptake, but enhanced P uptake and movement within the plant. Previous research has provided that the increase in plant growth due to P applications results in a dilution of Zn (Kisko et al. 2015), but later research noted that this plant growth may be related to the interactions between Zn and N within the plant tissue (Xue et al. 2021) because of the enzymatic reactions Zn performs in the plant N-cycle. Specifically, N increases the plant growth more than P, but does not decrease the concentration of Zn within the plant tissue. It is suggested that there are some physiological processes involved in this effect, like high levels of P inhibiting translocation of Zn from roots to metabolic sites in the leaves of cotton, orchids, and maize as well as many other horticultural plants (Cakmak & Marschner 1986, Ova et al. 2015, Novais et al. 2016, Zhang et al. 2017). These Zn deficiencies lead to accumulation of P in

plants because they then lose control over P absorption mechanisms; the inverse is also true (Kisko et al. 2015). This is specific to Zn deficiency and not in other mineral deficiencies such as Fe, Mn, or Cu. Research by Santos et al. (2021) also suggests that excess P in cotton plants reduced the Zn-shoot ratio, producing reduced growth, and decreased photosynthesis-related parameters.

Excess P concentrations can also affect plant color, potentially causing chlorosis and necrosis of leaves, limiting growth through physiological and morphological disruption, and potentially affecting the formation and distribution of mycorrhizae, with plant root systems (De Kock & Wallace 1965, Shen et al. 2011). Many crops can thrive in areas with high P. Depending on soil properties and crop type excess available soil P can potentially be detrimental in East Texas if not managed properly (Gascho et al. 2006). Conversion of these lands to loblolly pine plantations is often problematic, resulting in poor seedling survival and abnormal growth.

The overall purpose of this study is to provide an assessment of the environmental impacts of long-term poultry litter application on loblolly pine seedlings within East Texas, in relation to soil P content. It was also performed to determine if excessive soil P causes abnormal growth and survival, and what physiological process may contribute to those defects.

## OBJECTIVES

The purpose of this project is to determine the impact of excess available soil P on loblolly pine (*Pinus taeda* L.) seedlings in terms of physical growth and plant nutrition. More specifically, the objectives of this study are:

1. Determine if excess available soil P effects the health and development of loblolly pine seedlings.
2. Determine the nutritional relationship between excess available soil P and loblolly pine seedlings.
3. Determine the nutritional relationship between excess soil P and micronutrients (Zn and Fe) uptake in loblolly pine.
4. Determine if loblolly pine mycorrhizal development is affected by excess soil P.
5. Determine if loblolly pine foliage coloring is affected by excess soil P.

# LITERATURE REVIEW

## East Texas Loblolly Pine Production

Forests within the eastern portion of Texas are part of the southern forest region of the United States, known as Region 8. States within this region include Virginia, Kentucky, Tennessee, North Carolina, South Carolina, Georgia, Alabama, Mississippi, Arkansas, Louisiana, southeastern Oklahoma, and the Eastern most part of Texas. Loblolly pine is the dominant pine species within Region 8 making up over 45% (13.4 million ha) of the available pine volume (Schultz 1999). The area of focus of this study is in the Piney Woods of East Texas, covering approximately 14 million hectares of coniferous terrestrial forest (Weakley et al. 2009). Large portions of the native forest have been harvested in recent decades and replaced by loblolly pine plantations due to its high adaptability and rapid growth. These stands are man-made ecological communities used to increase timber production per unit land area and time though crop manipulation, and implementation of biotic and abiotic improvements. Additionally, peripheral land around the Piney Woods is largely made up of agricultural pastureland and in

recent decades considerable amounts of this pastureland have been planted to loblolly pine.

By 2030, pine pulpwood demand is projected to increase by 40% since the 1987 US Forest Service assessment and the demand for pine sawtimber volume is expected to increase by 12%. Much of this pastureland that was fertilized with poultry litter for decades before being transitioned into pine plantations (Harwell & Dangerfield 1991).

## Loblolly Pine Physiology

### Foliar and Root Nutrient Content

Foliar and root nutrient content can provide important information as to what nutrients have been taken up by the plant from the soil. Seasonal trends are not readily predictable due to difference in site characteristics and variable weather (Adams et al. 1987) and in most cases foliar nutrient content is less telling of fertilizer-induced changes than root nutrient concentrations. Though, there is little research relating to the nutrient content of roots to the nutrient deficiencies of loblolly pine. For example, Adams et al. (1987) noted that foliage is more sensitive to N deficiencies, while roots are more sensitive to P deficiencies. At or below

nutrient critical range, the tree will develop morphological responses such as chlorosis and necrosis of the fascicle, as well as physiological or morphological defects during development (Turner et al. 1977). The majority of tree health indicators will depend on if it is at or below the optimum nutrient requirements and the compounding effect of multiple stressors.

## Mycorrhizae

Mycorrhizae are an association between roots and fungi through a symbiotic, or mildly pathogenic, relationship. Fungi colonize the root system of the host plant, providing increased surface area for water uptake and increased nutrient absorption. This increases available nutrient absorption and reduces water stress (Barnett & Brissette 1986, Bonfante & Genre 2010). In return the host plant provides carbohydrates formed through photosynthesis. There are anywhere from 200 to 1,000 species of fungi that form a mycorrhizal relationship with the root system of loblolly pine (Schultz 1997). Mycorrhizae colonizing loblolly pine are ectotrophic, forming a feltlike cover over the root itself and extending into the soil. This type of fungi does not penetrate the cells within the root but are intercellular. The hyphae will penetrate the root cortex into the intercellular space where there can be an exchange of nutrients and minerals between plant and fungi (Reddy et al.

2013). External mycelia vary among species. Long roots with rapid growth generally do not have mycorrhizal associations but instead have mycorrhizae branched off the growing tip to form bifurcated root tips that are quickly inoculated (Taylor et al. 2014).

The average containerized seedling is grown in a medium meant to optimize growth utilizing high fertility. Conditions like this inhibit the growth of mycorrhizae and introduction to areas with low fertility will facilitate inoculation (Barnett & Brissette 1986). For example, Reid et al. (1983) found that after inoculation, 9% of all roots on a two-month-old loblolly pine seedling were mycorrhizal. By four and ten months, 40% and 73% of all roots were mycorrhizal, respectively. Inoculated trees benefit from increased survival time of short roots from inorganic and organic soil toxins, soil acidity, and high soil temperatures (Reid at el. 1983).

## Indicators of Seedling Quality

The main indicators utilized for seedling quality are morphological features, such as root:shoot ratio, root collar diameter, shoot height, and plant tissue color (Thompson 1985, Barden 1987, Binotto et al. 2010, Mohamed 2013, Lin et al. 2019). Seedling size may therefore be an indicator for quality, but it should not be relied upon for consistency. Any correlations between seedling size and survivability are mixed due to the strong correlation between site characteristics and seedling size. South et al. (1985) showed significant effects of size on survival, height, and volume of seedlings exceeding three years of age. The use of phenotypic traits and plant indices can be used to express plant efficiency benchmarks when subject to environmental factors, such as excess soil P.

The Sturdiness quotient (SQ) index is a nondestructive method of evaluating a seedlings quality with a comparison of seedling height (SH) divided by the root collar diameter (RCD) (Lin et al. 2019). The SQ ratio of height to diameter is meant to express the vigor of each seedling or robustness (Thompson 1985, Lin et al. 2019). Values calculated less than six are ideal and indicate a sturdy plant that has a higher rate of survival.

$$SQ = \frac{SH\ (cm)}{RCD\ (mm)} \qquad \text{(Eq. 1)}$$

The Dickson seedling quality index (DQI) is another tool to evaluate a seedlings quality based on the nutrient environment within which the seedling is grown (Eq. 1). It utilizes a number of possible combinations of morphological variables as a way to predict field performance of pine seedlings (Thompson 1985). It is also capable of predicting quality based on the nutrient environment (soil fertility) that the seedlings are grown in.

This is a function of total dry matter (TDM), shoot height (SH), root collar diameter (RCD), shoot dry matter (SDM) – the sum of the stem base dry matter and leaf dry matter – and root dry matter (RDM) (Binotto et al. 2010). DQI is expressed by Equation 1 below:

$$DQI = \frac{TDM\ (g)}{\frac{SH\ (cm)}{RCD\ (mm)} + \frac{SDM\ (g)}{RDM\ (g)}} \quad \text{(Eq. 2)}$$

## Poultry Litter

Poultry litter is made up of organic waste and the main quantity is sourced from chicken and turkey operations primarily in CAFOs. It also contains spilled feed, feathers, supplements, and bedding materials (Ashworth et al. 2020). It is a

low-cost alternative to inorganic chemical fertilizers widely available in poultry producing areas and has the potential to increase soil organic matter and nutrient availability (Bryant et al. 2021). Gaskin & Harris (2017) report that a ton of poultry litter on average contains 24.9 kg N, 25.9 kg P and 21.3 kg K, although the nutrient content can be highly variable and is influenced by factors such as litter age, poultry diet, amount of bedding, storage, and method of application (Keena 2021). Ashworth et al. (2020) noted that litter can vary in its nutrient content up to 30% due to the differences among broilers. When dealing with nutrient management, the content of each independent operation should be assessed. It should also be noted that very few studies have looked at poultry litter application to Gulf Coastal Plains soils. Generally, it is not recommended that pine plantations be fertilized in areas with sandy soils since water deficit limits productivity (Jokela & Long 2012) but private landowners frequently fertilize to increase timber volume growth and pine straw production (Minogue et al. 2012).

The southeastern US is the leading region in poultry production with approximately 6.6 billion chickens raised per year (USDA 2004) and 6.3 Tg $yr^{-1}$ of poultry litter as byproduct (Blazier et al. 2008). Poultry litter contains a significant concentration of important plant nutrients, mainly N and P (Hansen et al. 2002). Land fertilized with poultry litter has been found to produce significant volume increase in loblolly pine and has opened an expansive potential for poultry litter use. It is not economically viable to transport poultry litter far from production. As

a result, it is typically applied to pastureland close to the poultry production due to several barriers including: limited availability due to time constraints, risk due to variable nutrient constraints, lack of research regarding performance under various crop management systems, and most importantly transportations costs (Bryant et al. 2021). Short distance application often leads to over application to nearby pastures and forest stands in close proximity to poultry-production facilities. These practices lead to increased concentrations of P, K, Ca, Mg, Cu, and Zn within soils after an extended period of application (Kingery et al. 1994, Gaskin & Harris 2017). Watershed pollution is often associated with nutrient-saturation in these soils (Sharpley et al. 1987). It has also been found that determining the nutrient content before application can help reduce P pooling, runoff, and soil nutrient surplus.

Poultry litter is frequently applied to meet the N nutrient limitation, leading to a surplus of P that then builds up in the soil, which can move into nearby waterbodies where the chance of eutrophication is increased. Only approximately 2% of applied P (in particulate and dissolved forms) is lost to surface runoff. The rest is absorbed by soil minerals and accumulated to create 'legacy phosphorus' (legacy P) (Zhu et al. 2018). Legacy P refers to the left-behind soil P surplus in managed soils from over application that does not lead to increased production (Lou et al. 2018, Zhu et al. 2018, Pavinato et al. 2020). Saturation and leaching of P is most likely to happen in areas with high fertility and sandy soils (Kleinman et

al. 2015) similar to pine plantations of East Texas. These forests soils also have a high capacity for retention of nutrients due to high biomass and soil organic matter content (Will et al. 2006). There is still speculation as to how much legacy P there actually is in the soils. Areas with long-term exposure to poultry litter have seen a large portion – greater than 70% – of a surplus of P remaining in the soil (Pavinato et al. 2020).

## Poultry Litter Fertilization

Within the United States forest land of the Pacific Northwest and the southeast are the primary regions of fertilizer usage. By the mid-1980s around 101,000 ha of forested land had been fertilized annually, and by the mid-1990s this average increased to 150,000 ha of land fertilized annually (Evans 2000). Whether a stand requires fertilization is dependent on several factors such as nutritional deficiencies, whether the vegetation is responsive to the proposed added nutrients, and whether the area is large enough – at least 40 acres – to be managed operationally (Dickens et al. 2003). In addition, consideration must be given to the interaction between the fertilization process and the forest production system. For example, certain results can be expected when applying specific N-P-K formulation ratios to a stand, but differences between site, species, soil moisture, and age – as well as any other related processes - can provide entirely different results given the same N-P-K

formula. Any improvement within the overall optimization of stand productivity and development will come from refinement in maintenance and general nutrient availability (Wells & Allen 1985). These developments depend on the nutritional status of the site, needs and potential responsiveness of the vegetation (Jokela et al. 1991).

Sites are typically evaluated for any nutrient deficiencies by foliar sampling and analysis. Some areas within East Texas are termed marginal and experience nutritional deficit. Disregarding productive sites, N and P are generally the most limiting nutrients in terms of growth in the region (Jokela & Long 2012). Within loblolly pine stands, a combination of N and P fertilization has been found to be more effective than fertilization with strictly P. Within the stand, N from mineral fertilizer is typically only available for 1 – 2 years post fertilization, while P has been found to typically last the entire rotation on previously deficit sites and tends to add to the sites nutrient budget (Wells & Allen 1985). Due to this, sites are typically fertilized based on the N need when applying poultry litter which can cause a surplus of other available nutrients within the soil (Ova et al. 2015). Nutrient release dynamics in the litter layer and the $O_i$ horizon are not significantly affected by fertilization. However, fertilization often stimulates microbial decomposers within the $O_e$ and $O_a$ horizons affecting the nutrient release dynamic, increasing decomposition rates (Bot & Benites 2005) and is also a great influencer

for increasing growth and development in relation to economic desirability (Dickens et al. 2003, Albaugh et al. 2004).

## Phosphorus

Total P is made up of three separate classifications, all originating from both organic and inorganic forms: plant-available P, labile P, and non-labile P (Mengel et al. 2001, Costa et al. 2016). Plant-available P, or solution P, is made up of mainly inorganic P that is dissolved in a water – soil solution (Prasad & Chakraborty 2019). This portion of the soils P is a relatively small portion of total P but is the form mainly taken up by plants. Another portion of soil P that is available to plants is classified as labile P, comprising of inorganic P forms adsorbed to silicate clays, carbonates, Fe, and Al (hydro)oxides (Grenon et al. 2021). This portion of active soil P is somewhat slow to release but is still available to plants. It typically makes up only 1% to 5% of P in soils (Prasad & Chakraborty 2019). Over time, P solubility potential will decrease after fertilization. Absorbed P will begin to precipitate on mineral surfaces such as Fe and Al oxides and Ca phosphates (Mengel et al. 2001). Fixed P makes up the largest portion within the soil and is largely unavailable to plants (Mengel at al. 2001). Release of fixed P to active P occurs very slowly over time. These three P

classifications may correspond in equilibrium, replenishing each other through time and the applications of manure or fertilizers add to the plant-available P to support plant needs during primary growth stages (Prasad & Chakraborty 2019). Phosphorus uptake is mainly sourced from the soil by plant roots and associated mycorrhizae, but many factors, such as the chemistry and composition of the soil matrix can hinder absorption (Morgan & Connolly 2013). Other influencing factors are related to soil properties such as soil moisture, pH, and porosity.

## Micronutrients

Three essential macro- and micro- nutrients of focus in this study, necessary for the survival and development of all organisms are inorganic phosphate (Pi), Zn, and Fe (Xie et al. 2019). They are relatively inaccessible by vegetation due to low solubility and soil immobilization. Because of this, Zn and Fe deficiencies are common in agricultural settings and in recent decades has become a common concern due to the push for higher yields (Neset & Cordell 2012, Shahzad et al. 2014, Ova et al. 2015). Deficiencies such as this are common in sandy soils and soils with high P content (Drissi et al. 2015). There are multiple explanations for the Zn-P relationship, 1) dilution of Zn due to enhanced plant growth from excess P, 2) lowered translocation from root to shoot from P interference, and 3) reduction

of Zn availability due to the Zn-P interaction in the soil (Lee & Doolittle 2004, Drissi et al. 2015, Xie et al. 2019). Some studies have found that the Zn-P relationship is dependent on plant mycorrhizae development (Ova et al. 2015) and others have reported that application of P in excess increases growth in vegetation, but causes visual symptoms of Zn deficiency (De Kock & Wallace 1965, Shi et al. 2018, Xie et al. 2019). Research has tested the associated effect of differing rates of Zn and P supply on growth, nutrient content, and total biomass (Nguyen et al. 2019, Pongrac et al. 2020). Iron and Zn uptake is mainly sourced from the rhizosphere. Since East TX has predominantly acidic soils, Fe is in a freed state and is readily available for plant uptake (Morrissey & Guerinot 2009). Much like Fe, Zn uptake is mainly facilitated by fine roots and its general availability in the soil (Welch & Graham 2005). Zinc is more mobile in acidic soils due to the insoluble complexes that form in alkaline soils (Barber 1995).

## Munsell Classification System

The Munsell color classification system is frequently used as a descriptor, though with the application of logistic regression it may be used to visibly evaluate plant tissue though color. Color is determined by reflected frequencies that are visible to the human eye. The three dominant frequencies that can affect the human

eye are red, green, and blue (Norris 1977). As a means to quantify color, the Munsell classification system was developed by Albert Munsell in 1905 and is an approximately spherical representation of colors organized into unique spatial locations (Ruck & Brown 2015).

Color can also be differentiated by three variables: hue, value and chroma (Ruck & Brown 2015). Hue is the distinction between the five principle chromatic colors such as red (R), yellow (Y), green (G), blue (B), and purple (P). This is the quality where the observer is aware of the differing radiant energy wavelengths being reflected (Petryshyn, 1967). The full Munsell system has 40 hues with their own alphanumeric description. Value refers to the lightness that is dominant and varies numerically from 0 (black) to 10 (white) (Ruck and Brown 2015), and finally, chroma represents the purity of the hue. It can range from neutral to strong colors (0 to 12 respectively) (Zelenak 1995). Figure 1 details a three-dimensional representation of the Munsell classification system.

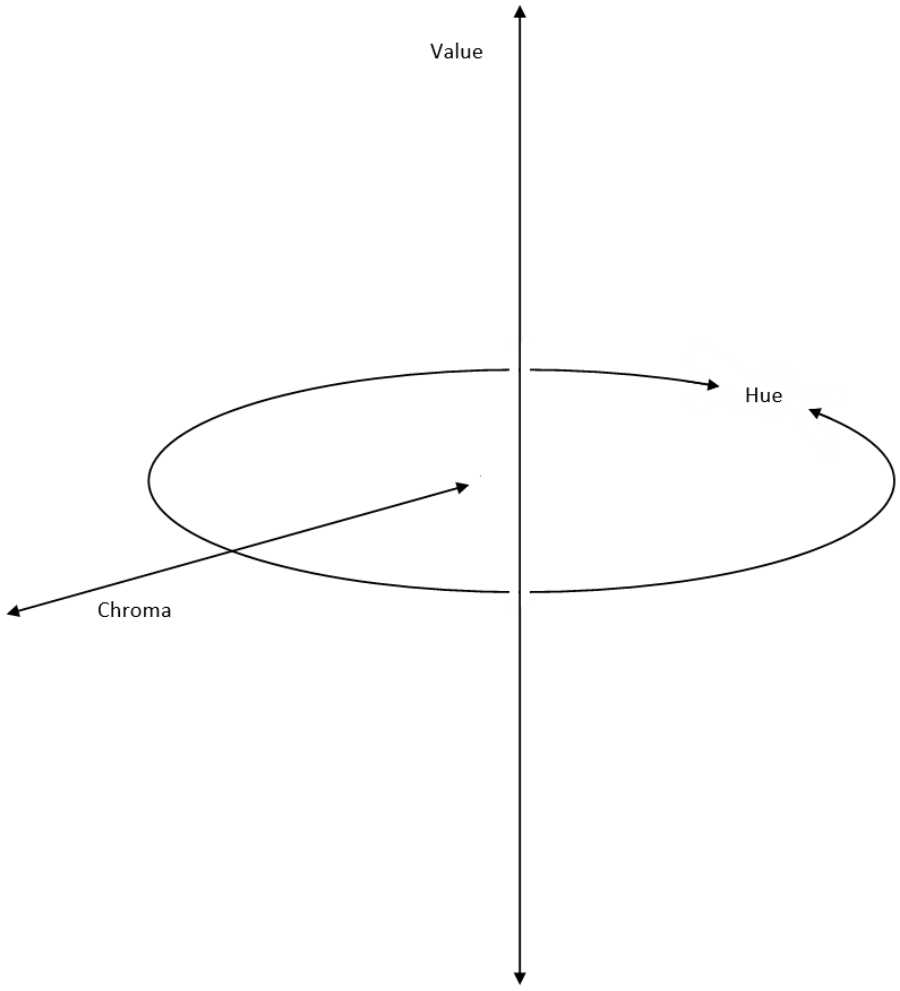


Figure 1. Arrangement of Hue, Value, and Chroma within the Munsell three-dimensional color space.

Classically, researchers record the Munsell colors and then report data in a descriptive manner. They usually report the most common color present or range visible on a particular attribute (Norris 1977, Ruck & Brown 2015). Scarcely, is an analysis done on a data set. D'Andrade & Romney (2003) reported that since the Munsell color space is inherently a three-dimensional system it can be represented as a standard Cartesian coordinate. Color variables were converted to align with this 3D system. Ruck & Brown (2015) suggested that Munsell color measurements can be utilized to create distinct groups to exhibit statistically significant differences in color.

Munsell color transformation within this analysis followed the research of D'Andrade & Romney (2003) as well as Ruck & Brown (2015). The Munsell color data is transformed into Cartesian coordinates. Firstly, hue is converted into angles followed its 3-dimensional orientation within the color space. Consider the 40 hue designation pages within a color book fanned out in a circle around the binding. If each page is equally distant from the next, they would be situated in 9° intervals. The hue 5R was chosen as the origin at 0°. Following this conversion all degree values were converted to radians to help ease calculations in Microsoft Excel.

Chroma is the distance the color is from the central axis. Paired with the angle calculated from the Hue, a unique point within the plane can be described. The

remaining coordinate is the Value of the color that describes the height of the point in the 3-dimensional space. These points are then converted to Cartesian coordinates using the following equations:

$$x = \sin(Hue) * (Chroma) \quad \text{(Eq. 3)}$$

$$y = \cos(Hue) * (Chroma) \quad \text{(Eq. 4)}$$

$$z = Value \quad \text{(Eq. 5)}$$

Based on this research, logistic regression can be utilized to test hypotheses posed with color data sets regarding archeological ceramics and the separation of named color samples. Distinctions such as this could possibly be utilized for plant tissue color analysis to separate color and visible plant tissue nutrient deficiencies, supplying a means of visible quantification. Another factor for consideration is the waxy cuticle surrounding the needle and how it could affect color collection. Removal of the cuticle may change the color.

# METHODS

## POT STUDY

### Study Area

The outdoor study was conducted at Stephen F. Austin State University (SFASU) adjacent to the Arthur Temple College of Forestry and Agriculture (ATCOFA) greenhouse in Nacogdoches, Texas. Annual precipitation averaged 1250 mm, and was well distributed throughout the year, though there were prevalent dry conditions in June 2021. The seasonal summer temperature, July – August, averaged 26.8° C, and the seasonal winter temperature, December – February, averaged 9.4° C. The site is nearly level, and all pots were elevated by 12 cm to prevent contact with the ground. The approximate site latitude is 31°34’24” N and longitude at 94°38’46” W. Figure 2 details the site location in reference to surrounding counties.

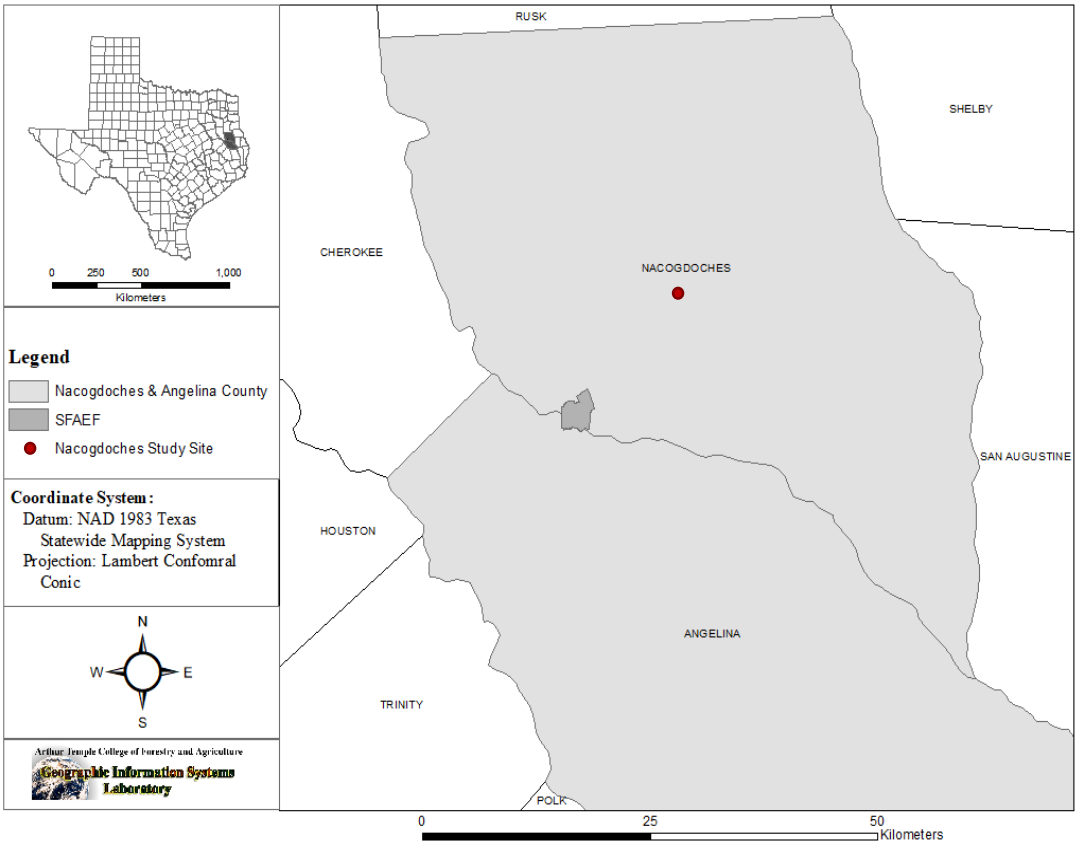


Figure 2. Location of the outdoor pot study site for growth response to variable P concentrations in Nacogdoches, Texas.

## Experimental Design

This portion of the study utilized bare-root loblolly pine seedlings (AG – 615) from the ArborGen Bullard Nursery in Bullard, Texas. There was a total of six treatments, with eight replications per treatment. Figure 3 details the pot placement and the approximate dimensions of the design. Triple superphosphate (0-46-0), utilizing $H_2PO_4^-$, was used to simulate different P concentrations. Fertilizer P content was calculated as 20.24% P by weight of triple superphosphate. The treatments in this study consisted of (P0) control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$ of P to potting media. Applicable values for fertilizer treatment rates were calculated using Equation 6. Media volume (MV) was 7100 $cm^3$ per pot. Potting media bulk density (Db) was determined after the potting media was created, using the oven-dried weight (60°C).

$$\frac{MV\ (cm^3)}{} \times \frac{Db\ (g\ soil)}{(cm^3)} \times \frac{Treatment\ (mg\ fert)}{(kg\ soil)} \times \frac{(g\ fert)}{Fertilizer\ P\ \%\ (g\ P)} \times \frac{(g)}{1000\ (mg)} \times \frac{(kg)}{1000\ (g)} \quad \text{(Eq. 6)}$$

Black plastic pots used measured 19.5 cm by 31.75 cm and held a potting media comprised of composted pine bark fines, Berger bark mix, and all-purpose sand (2:1:1 v/v). Each seedling pot was lined at the bottom with approximately 16 g of Poly-fil (Fairfield™ Processing, Danbury, CT) to prevent loss of potting media and fertilizer through the drainage holes.

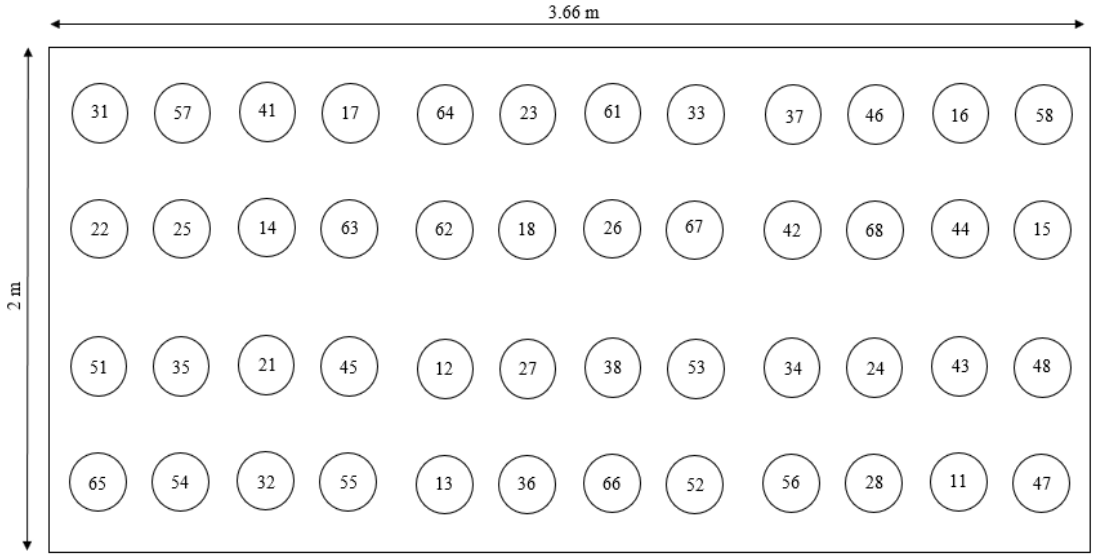


Figure 3. Randomized single representative layout for the outdoor pot study. Each pot is labeled with a value that represents the treatment (the first value; (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$) and the replication (the second value).

It has been well established that after phosphate fertilizer application, the phosphate affects the initial media pH to fluctuate within low acidic ranges after the first week of application (Saunders 1958). After setup with the treatments all pots received the first watering without seedlings to facilitate for any pH fluctuation and were left for seven days for the pH to stabilize. Seedlings were planted following the Natural Resource Conservation Service (NRCS) seedling planting guidelines. After planting, all seedlings were outfitted with a 'tree collar' (Figure 4) of white translucent Mylar (Dupont Tejin Films™, Chester, VA) (Biaxially-oriented polyethylene terephthalate) to keep out precipitation, but still allow for gas

exchange. The tree collars were fabricated using six mil (0.015 cm) Mylar and had a circumference of 30.48 cm. The opening for the shoot was variable depending on the diameter of the seedling. All seedlings at the start of the study were given starter nutrient treatments of 1.25 g of Osmocote (18-6-12) and 0.10 g of Urea (46-0-0) to meet base nutrient requirements. Initial measurements of height, root collar diameter, and Munsell color for greens (Hue, Value, Chroma) were recorded at the time of planting. All seedlings over the course of the study received the same watering pattern, wetting the potting media to as needed, but with minimal drainage.

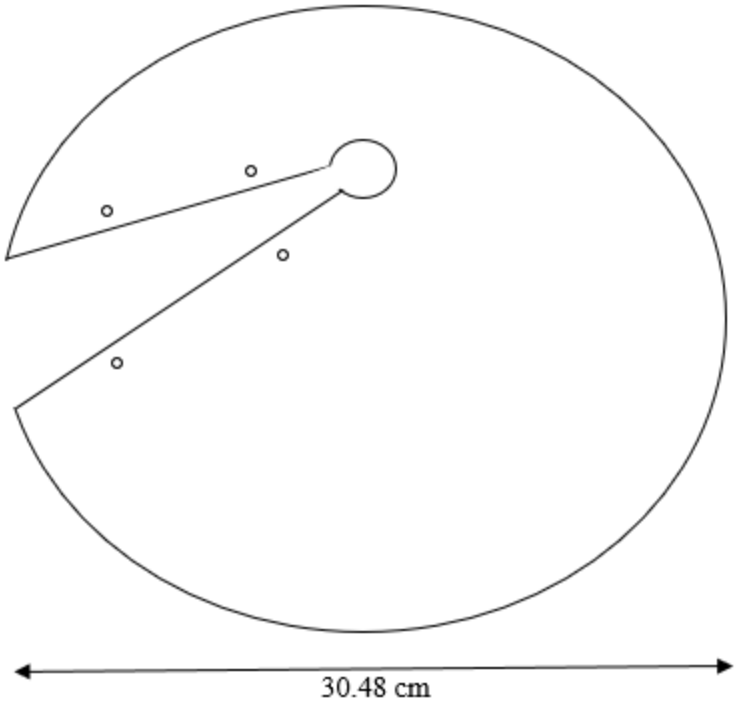


Figure 4. Tree collar shape design made of 6 mil opaque white Mylar. Collars are fastened with brass metal brads to allow easy removal during monthly measurements.

## DATA PROCESSING

### Data Procedures

### Measurements

Outdoor Pot Study

Measurements were taken each month the study was active. The following phenotypic measurements for each seedling were recorded:

1) Diameter (nearest 0.01 mm), measured twice at right angles using a Control Company (Traceable®, Webster, TX) digital caliper;
2) Shoot height, measure as height (cm) above the soil;
3) Munsell color for plant tissue, measuring the hue, value, and chroma of each seedling and making note if the seedling has any necrotic tips or of seedling mortality;

The pH was recorded at the start and termination of the study. After the first growing season all seedlings were collected, photographed, and analyzed for the following phenotypic measurements for each seedling:

1) Shoot height (cm), the measure of height above the soil from root collar to terminal bud;

2) Diameter (nearest 0.01 mm), measured using a Control Company digital caliper;
3) Root:Shoot (R:S), measured as the shoot dry mass (g) divided by the root dry mass (g) of the seedling;
4) Sturdiness quotient (SQ), calculated by dividing plant height by diameter;
5) Munsell color for plant tissue, measuring the hue, value, and chroma of each seedling and making note if the seedling had necrotic tips;
    a. Both the moist plant tissue color was recorded with the cuticle intact, and a dried ground color was recorded with cuticle broken;
6) Average branch length (cm), measured average length of all main branches on each seedling;
7) Average fascicle length (cm), measured average length of ten mature fascicles on each seedling;
8) Dickson Quality Index (DQI), measured by the seedlings total dry mass divided by the total sum of the SQ and R:S;

After data collection all needles and fascicles were removed. Root systems were stored at 4°C until they were analyzed. All root systems were washed to remove adhered potting media and photographed for comparative analysis. Five root segments per seedling were removed and counted for ectomycorrhizal root tips (EMRT) along bifurcated fungal root tips. The remaining root systems were separated from portions that cannot grow mycorrhizae for later analysis.

All portions of each seedling were placed into appropriately labeled paper bags and oven-dried at 60°C until they reached a constant weight. Once a constant weight was achieved, the following dry weights were obtained per seedling:

1) Total shoot dry weight (nearest 0.01 g)
2) Foliar dry weight (nearest 0.01 g)
3) Total root dry weight (nearest 0.01 g)
4) Tap root dry weight (nearest 0.01 g)
5) Lateral root dry weight (nearest 0.01 g)
6) Measured root segment dry weight (nearest 0.01 g)

The dried weight of the full root system was ratioed against the measured root segments using Equation 6 to determine an estimate of EMRT per seedling.

$$EMRT\ Estimate = \frac{\big(total\ root\ dry\ wt. - tap\ root\ dry\ wt.\ (g)\big)(EMRT\ )}{measured\ root\ segment\ dry\ wt.\ (g)} \quad \text{(Eq. 7)}$$

After the fascicles were dried and weighed, each sample was ground and send to the SFASU Soil Testing Laboratory for a full nutrient analysis, including: N, P, K, Mg, S, Na, Mn, Zn, Fe, Cu, Al, Mo, B, As, Ni, and Ca.

## Statistical Analysis

Seedling quality for the pot study was analyzed using both an analysis of covariance (ANCOVA) and analysis of variance (ANOVA) to compare each treatment level for each parameter. Data was analyzed through the Statistical Analysis System (SAS 9.4 (32 bit)) by the SAS Institute. Significant values were set at a significance level of 0.05 ($p = 0.05$). To help determine variable differences among treatments the Tukey post-hoc test was performed as an all pairwise comparison to help compare the means of all treatments to one another.

Regression analysis was used to examine the relationship between different growth yield parameters, nutrient uptake, and treatment levels of triple superphosphate. Several models were tested and gauged based off of their goodness of fit criteria of normal distribution, equal variance, and independence.

Munsell color analysis was conducted utilizing a logistic regression by transforming color data into cartesian coordinates. Cartesian coordinate conversion was conducted using equations 3, 4, and 5. The spatial representation of the Hue, Value, and Chroma was used as an evaluation tool to assess plant tissue through color.

## RESULTS AND DISCUSSION

*Pinus taeda* L. seedlings presented variable responses to varying levels of P. These responses were evident in plant growth dynamics between both initial and final measurements, nutrient uptake, and needle color. Mortality was not considered as no seedlings were lost during the first growing season. The six treatment levels utilized in this study were labelled as follows: control = P0, 250 mg $kg^{-1}$ = P1, 500 mg $kg^{-1}$ = P2, 750 mg $kg^{-1}$ = P3, 1000 mg $kg^{-1}$ = P4, and 1250 mg $kg^{-1}$ = P5.

### Height and Diameter Growth

Plant growth attributes such as height and diameter growth were measured prior to treatment application and following the first growing season. Seedling volume was calculated at the end of the first growing season. Table 1 shows the mean height growth, diameter growth, and volume of all seedlings by treatment following the first growing season.

Table 1. Mean seedling height (cm) and diameter (mm) per treatment after the first growing season.

| **Treatment** (mg P $kg^{-1}$ media) | **Parameters** | | |
|---|---|---|---|
| | **Height** (cm) | **Diameter** (mm) | **Volume** ($cm^3$) |
| **(P0) Control** | $14.73^{b}$ | $2.93^{b}$ | $21.91^{b}$ |
| **(P1) 250** | $19.26^{ab}$ | $4.08^{ab}$ | $30.14^{ab}$ |
| **(P2) 500** | $18.75^{ab}$ | $3.72^{ab}$ | $31.89^{ab}$ |
| **(P3) 750** | $23.04^{a}$ | $4.25^{ab}$ | $34.22^{a}$ |
| **(P4) 1000** | $22.75^{a}$ | $4.91^{a}$ | $39.98^{a}$ |
| **(P5) 1250** | $22.53^{a}$ | $4.74^{b}$ | $36.71^{a}$ |

Means in a column followed by the same letter had no significant difference at the 0.05 level. Mean comparisons were determined by the Tukey post hoc test.

Height growth over one growing season for six treatments, P0, P1, P2, P3, P4, and P5, was significant ($p < 0.05$) for treatment effect (Table 2). Height growth was highest in P4 and lowest in P0 (Table 1), means ranging from 17.73 cm to 22.75 cm. Diameter growth over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 2). Diameter growth was highest in P4 and lowest in P0 (Table 1), means ranging from 2.93 mm to 4.91 mm. Final volume over one growing season for six treatments was significant ($P < 0.05$) for treatment effect (Table 2). Mean volume was highest for P4 and lowest in P0 (Table 1), means ranging from 21.91 $cm^3$ to 39.98 $cm^3$.

Adjustment for multiple comparisons from the Tukey post-hoc test was performed in tandem with an ANCOVA and reported that the relationship of height growth between

the following treatments have strong relations, or hold significance: P0 v. P3 ($p = 0.0117$), P0 v. P4 ($p = 0.0163$), and P0 v. P5 ($p = 0.0210$) (Table 3 & Figure 7a). Adjustment for multiple comparisons from the Tukey post-hoc test was performed in tandem with an ANCOVA and reported that the relationship of diameter growth between the following treatments has strong relations, or hold significance: P0 v. P4 ($p = 0.0022$), and P0 v. P5 ($p = 0.0060$) (Table 4 & Figure 7b). Mean volume was had significant among treatments P0 v. P4 ($p = 0.0006$) and P0 v. P5 ($p = 0.0070$) (Table 5). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 20a-c & 21a-c).

Table 2. Height, diameter, and volume growth significance of interaction by treatment after one growing season utilizing Type III SS.

| **Parameter** | **Source** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Height | Treatment | 423.30 | 84.661 | 3.86 | 0.006 |
| Diameter | Treatment | 20.84 | 4.1671 | 4.54 | 0.021 |
| Volume | Treatment | 1558.33 | 311.6661 | 4.95 | 0.012 |

Table 3. Tukey post-hoc test probability for P concentration to affect height growth treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Height | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | 0.3951 | 0.5281 | 0.0117 | 0.0163 | 0.0210 | |
| **(P1) 250** | 0.3951 | | 0.9999 | 0.5960 | 0.6733 | 0.7312 |
| **(P2) 500** | 0.5281 | 0.9999 | | 0.4584 | 0.5349 | 0.5960 |
| **(P3) 750** | 0.0117 | 0.5960 | 0.4584 | | 1.0000 | 0.9999 |
| **(P4) 1000** | 0.0163 | 0.6733 | 0.5349 | 1.0000 | | 1.0000 |
| **(P5) 1250** | 0.0210 | 0.7312 | 0.5960 | 0.9999 | 1.0000 | |

Table 4. Tukey post-hoc test probability for P concentration to affect diameter growth treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Diameter | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | 0.1789 | 0.5818 | 0.0849 | 0.0022 | 0.0060 | |
| **(P1) 250** | 0.1789 | | 0.9713 | 0.9992 | 0.5271 | 0.7352 |
| **(P2) 500** | 0.5818 | 0.9713 | | 0.8684 | 0.1509 | 0.2806 |
| **(P3) 750** | 0.0849 | 0.9992 | 0.8684 | | 0.7480 | 0.9053 |
| **(P4) 1000** | 0.0022 | 0.5271 | 0.1509 | 0.7480 | | 0.9994 |
| **(P5) 1250** | 0.0060 | 0.7352 | 0.2806 | 0.9053 | 0.9994 | |

Table 5. Tukey post-hoc test probability for P concentration to affect volume growth treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Volume | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | 0.3196 | 0.1426 | 0.0375 | 0.0006 | 0.0070 | 0.0070 |
| **(P1) 250** | 0.3196 | | 0.9977 | 0.9060 | 0.1538 | 0.5684 |
| **(P2) 500** | 0.1426 | 0.9977 | | 0.9914 | 0.3391 | 0.8278 |
| **(P3) 750** | 0.0375 | 0.9060 | 0.9914 | | 0.6962 | 0.9884 |
| **(P4) 1000** | 0.0006 | 0.1538 | 0.3391 | 0.6962 | | 0.9614 |
| **(P5) 1250** | 0.0070 | 0.5684 | 0.8278 | 0.9884 | 0.9614 | |

Plant height growth, diameter growth, and volume are a base genetic characteristic of species but can be influenced through crop management, such as fertilization. Seedling growth increased in height and diameter the higher the P treatment levels (Tables 2 & Figures 6a & 6b). Maximum plant height was 55 cm in P4 and was lowest, 37 cm, in P0. Similarly, diameter growth was highest in P4 at 10.49 mm and lowest in P0 at 5.29 mm. Mean volume between treatments showed no real variation other than between P1 and P4, though trends increased as treatment dose increased. Trends dropped after P4 and mean height, diameter, and volume decreases in the highest treatment, suggesting that productivity drops in higher levels of excess soil P (Table 1). Throughout the study all seedlings were provided with the same base nutrients, independent of the phosphorus levels. Initial to final growth throughout the first growing season can be attributed to the application of P. Within this study parameters like height

growth, diameter growth, and volume have contrasting trends when compared to the health of the seedlings as treatment dose increases. Contrary trends include foliar nutrient concentrations (Tables 24 & 25), deficiency symptoms (Tables 29 & 30), and plant color (Figure 5). Results may differ with an increased study timeline over multiple growing seasons.

## Needle and Branch Length

Plant morphological attributes such as needle and branch length were measured at the end of first growing season prior to destructive sampling of the seedlings. Table 5 shows the mean needle and branch length of all seedlings by treatment after the first growing season.

Table 6. Mean average needle length (cm) and branch length (cm) per treatment after the first growing season.

| **Treatment** (mg P $kg^{-1}$ media) | **Parameters** | |
|---|---|---|
| | **Mean Needle Length** (cm) | **Mean Branch Length** (cm) |
| **(P0) Control** | $12.61^{b}$ | $15.03^{b}$ |
| **(P1) 250** | $14.55^{ab}$ | $18.31^{ab}$ |
| **(P2) 500** | $12.65^{b}$ | $18.56^{ab}$ |
| **(P3) 750** | $14.06^{ab}$ | $19.28^{ab}$ |
| **(P4) 1000** | $14.88^{a}$ | $20.33^{a}$ |
| **(P5) 1250** | $14.67^{a}$ | $19.77^{ab}$ |

Means in a column followed by the same letter had no significant difference at the 0.05 level. Mean comparisons were determined by the Tukey post hoc test.

Mean needle length after one growing season for six treatments was significant ($p < 0.05$) for treatment effect. Final needle length measurements were highest in P4 and lowest in P0 (Table 5), means ranging from 12.61 cm to 14.88 cm. Mean branch length over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 6). Final branch length measurements were highest in P4 and lowest in P0 (Table 5), means ranging from 15.03 cm to 20.33.

Adjustment for multiple comparisons from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationship of needle length between the following treatments have strong relations, or hold significance: P0 v. P4 ($p = 0.0192$) and P0 v. P5 ($p = 0.0421$) (Table 7 & 7c). Adjustment for multiple comparisons from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationship of branch length between the following treatments have strong relations, or hold significance: P0 v. P4 ($p = 0.0210$) (Table 8 & Figure 7d). Model assumptions for both needle and branch length both follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 22a-c & 23a-c).

Table 7. Average total mean needle length and mean branch length significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Needle Length | Treatment | 41.767 | 8.353 | 4.58 | 0.002 |
| Branch Length | Treatment | 141.272 | 28.254 | 2.78 | 0.029 |

Table 8. Tukey post-hoc test probability for P concentration to affect mean needle length treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: Branch Length | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.326 | 0.251 | 0.103 | 0.021 | 0.051 |
| **(P1) 250** | 0.326 | | 1.000 | 0.990 | 0.801 | 0.940 |
| **(P2) 500** | 0.251 | 1.000 | | 0.997 | 0.874 | 0.973 |
| **(P3) 750** | 0.103 | 0.990 | 0.997 | | 0.986 | 1.000 |
| **(P4) 1000** | 0.021 | 0.801 | 0.874 | 0.986 | | 0.999 |
| **(P5) 1250** | 0.051 | 0.940 | 0.973 | 1.000 | 0.999 | |

Table 9. Tukey post-hoc test probability for P concentration to affect mean branch length treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: Needle Length | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.066 | 1.000 | 0.279 | 0.019 | 0.042 |
| **(P1) 250** | 0.066 | | 0.077 | 0.979 | 0.996 | 1.000 |
| **(P2) 500** | 1.000 | 0.077 | | 0.313 | 0.023 | 0.050 |
| **(P3) 750** | 0.279 | 0.979 | 0.313 | | 0.831 | 0.944 |
| **(P4) 1000** | 0.019 | 0.996 | 0.023 | 0.831 | | 1.000 |
| **(P5) 1250** | 0.042 | 1.000 | 0.050 | 0.944 | 1.000 | |

Similar to other growth patterns observed, both mean needle and branch length increased as treatment application increased (Table 6). Needles in the higher treatments (Table 30 & Figures 43-48) nearing the end of the first growing season developed a wilt, less turgor, and a more pronounced development of chlorosis of plant tissue and tip necrosis (Table 25). Deficiencies like chlorosis, necrosis, and stunted productivity are common symptoms of decreased or hindered Fe and Zn uptake (Millikan 1963, Smith & Mitchell 1977, Shahzad et al. 2014, Ova et al. 2015, Shi et al. 2018). These morphological developments can also be associated high foliar P nutrient content and its relation to Fe and Zn (Table 24 & Figure 12-19) where mechanisms like micronutrient dilution (De Kock & Wallace 1965) and uptake (Xie et al. 2019) are influenced by excess soil P. Some research reports that the full effect of Zn or Fe deficiency associated with shoot health in conifers does not fully develop until the second growing season (Bromley 2011).

## pH

Environmental conditions like the pH of the potting media were measured after the application of triple superphosphate and after the first growing season. Table 9 shows the mean pH of all seedlings pots per treatment prior to seedling planting and following experiment termination.

Table 10. Mean media pH per treatment after the first growing season.

| **Treatment** (mg P/kg media) | **Parameters** **Mean pH** |
|---|---|
| **(P0) Control** | 5.54 |
| **(P1) 250** | 5.54 |
| **(P2) 500** | 5.27 |
| **(P3) 750** | 5.41 |
| **(P4) 1000** | 5.46 |
| **(P5) 1250** | 5.43 |

Final pH after one growing season for the six treatments was not significant ($p > 0.05$) for treatment effect (Figure 10). Final mean pH was highest in P4 and lowest in P2, means ranging from 5.27 to 5.46 (Table 9). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figure 24a-c).

It has been established that phosphate retention in soils can cause change in the pH. This factor was taken into consideration at the beginning of the pot study. Research conducted by Saunders et al. (1958) suggested that differing phosphate fertilizers will have varying effects on the soil pH, and even a light top dressing is enough to

significantly change the pH. Research indicates that after P fertilization there is a stark initial decrease in pH one day after fertilization (Saunders et al. 1958), though the decrease is dependent on the type of phosphate fertilizer used. To avoid unwanted stressors, all potting media was fertilized and watered a week prior to planting. Average pH at the time of planting was 5.72. Final pH across all treatments showed no significance (Table 10) and was relatively stable at a somewhat acidic pH (Table 9), range varying by 0.15 units among treatments. Taking this into account, pH differences were not a factor on the differing growth or morphological development of the seedlings by treatment.

Table 11. pH significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III | Mean | F Value | Pr > F |
|---|---|---|---|---|---|
| pH | Treatment | 0.394 | 0.079 | 1.23 | 0.311 |

Table 12. Tukey post-hoc test probability for P concentration to affect pH treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: pH | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 1.000 | 0.308 | 0.909 | 0.990 | 0.962 |
| **(P1) 250** | 1.000 | | 0.294 | 0.898 | 0.987 | 0.956 |
| **(P2) 500** | 0.308 | 0.294 | | 0.887 | 0.676 | 0.796 |
| **(P3) 750** | 0.909 | 0.898 | 0.887 | | 0.999 | 1.000 |
| **(P4) 1000** | 0.990 | 0.987 | 0.676 | 0.999 | | 1.000 |
| **(P5) 1250** | 0.962 | 0.956 | 0.796 | 1.000 | 1.000 | |

## Total Dry Matter

Seedling quality determinates and plant growth attributes like TDM were calculated following the destructive sampling of the seedlings. Table 12 shows the mean TDM by treatment.

TDM over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 13). TDM was highest in P4 and lowest in P0 (Table 12), means ranging from 34.79 g to 51.41 g.

Adjustment for multiple comparison from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationship between the following treatments have strong relations, or hold significance: P0 v. P3 ($p = 0.013$), P0 v. P4 ($p = < 0.001$), P0 v. P5 ($p = 0.010$), P1 v. P4 ($p = 0.001$), P2 v. P4 ($p = 0.029$) (Table 14 & Figure 7f). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figure 25a-c).

TDM is not typically used as a grading method for seedling quality due to time restrictions and its destructive nature. Loblolly pine has been noted to have strong correlations between TDM and diameter (Switzer & Nelson 1963) and this holds true in this study (Figure 9) indicating a positive trend. Both variables correlate to the seedling survivability where the higher TDM and diameter are in ratio to another, the better the seedling subsistence (Thompson 1985). In general, seedlings with heavier dry weights

are more sought after if there is balance between the dry shoot and root weight. As mentioned, there are strong correlations between the control, lower treatments, and higher treatments, to TDM (Figure 14 & Figure 7f) showing an increase in growth productivity as treatment dose increases. Means for TDM show an increase in trend weight as treatment dose increases (Figure 9), through this does not speak for relative vigor and health characteristics of the seedling (Tables 24 & 29). Results may vary from an extended study timeframe.

Table 13. Total and mean dry weight (g), including shoot (g) and root (g) dry weights after the first growing season. Root-to-Shoot ratio, sturdiness quotient, and Dickson quality index mean calculations per treatment included.

| **Treatment** | **Dry Weight (g)** | | | **R:S ratio** | **SQ** | **DQI** |
|---|---|---|---|---|---|---|
| --(mg $kg^{-1}$)-- | **Total** | **Shoot** | **Root** | | | |
| **(P0) Control** | $34.79^{c}$ | $21.87^{c}$ | $12.93^{b}$ | 0.60 | 5.15 | $5.21^{b}$ |
| **(P1) 250** | $37.89^{bc}$ | $24.44^{bc}$ | $13.46^{ab}$ | 0.55 | 5.25 | $5.42^{b}$ |
| **(P2) 500** | $41.52^{bc}$ | $26.92^{bc}$ | $14.60^{ab}$ | 0.54 | 4.77 | $6.29^{ab}$ |
| **(P3) 750** | $45.64^{ab}$ | $30.36^{ab}$ | $15.29^{ab}$ | 0.51 | 5.47 | $6.15^{ab}$ |
| **(P4) 1000** | $51.41^{a}$ | $33.81^{a}$ | $17.59^{a}$ | 0.51 | 5.23 | $7.53^{a}$ |
| **(P5) 1250** | $45.90^{ab}$ | $29.69^{ab}$ | $16.21^{ab}$ | 0.55 | 5.24 | $6.40^{ab}$ |

Means in a column followed by the same letter had no significant difference at the 0.05 level. Mean comparisons were determined by the Tukey post hoc test.

Table 14. Total dry matter (g), shoot dry matter (g), root dry matter (g), and root-to-shoot ratio significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| TDM | Treatment | 1452.268 | 290.454 | 7.61 | <.0001 |
| SDM | Treatment | 748.152 | 149.630 | 9.42 | <.0001 |
| RDM | Treatment | 120.929 | 24.186 | 2.80 | 0.0287 |
| RS Ratio | Treatment | 0.043 | 0.009 | 1.16 | 0.343 |

Table 15. Tukey post-hoc test probability for P concentration to affect total dry matter treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: TDM | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.914 | 0.269 | 0.013 | <.0001 | 0.010 |
| **(P1) 250** | 0.914 | | 0.846 | 0.144 | 0.001 | 0.122 |
| **(P2) 500** | 0.269 | 0.846 | | 0.764 | 0.029 | 0.717 |
| **(P3) 750** | 0.013 | 0.144 | 0.764 | | 0.437 | 1.000 |
| **(P4) 1000** | <.0001 | 0.001 | 0.029 | 0.437 | | 0.487 |
| **(P5) 1250** | 0.010 | 0.122 | 0.717 | 1.000 | 0.487 | |

## Root and Shoot Dry Weight and Root-to-Shoot Ratio

Seedling quality determinates and plant growth attributes like the dry weight of the root and shoot, as well as the root-to-shoot ratio were calculated following the destructive sampling of the seedlings. Table 12 shows the mean dry shoot weight, dry root weight, and root-to-shoot-ratio.

SDM over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 13). SDM was highest P4 and lowest in P0 (Table 12), means ranging from 21.87 g to 33.81 g. RDM over one growing season for six treatments, P0, P1, P2, P3, P4, and P5 was significant ($p < 0.05$) for treatment effect (Table 13). RDM was highest in P4 and lowest in P0 (Table 12) with means ranging from 12.93 g to 17.59 g. RS ratio over one growing season for six treatments was not significant ($p > 0.05$) for treatment effect (Table 13). RS ratio was highest in the control and was lowest in P3 and P4 (Table 12), means ranging from 0.51 to 0.60.

Adjustment for multiple comparison from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationships of SDM between the following treatments have strong relations, or hold significance: P0 v. P3 ($p = 0.002$), P0 v. P4 ($p = < 0.0001$), P0 v. P5 ($p = 0.004$), P1 v. P4 ($p = 0.0004$), and P2 v. P4 ($p = 0.015$) (Table 15 & Figure 7g). Adjustment for multiple comparison from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationship of RDM between the following treatments have strong relations, or hold

significance: P0 v. P4 ($p = 0.031$) (Table 16 & Figure 7h). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 26a-c, 27a-c & 28a-c).

It is thought that the more shoot weight there is, the better the seedling will perform considering the RDM has a complementing weight (Thompson 1985). There is no significant difference in the RS ratio among treatments in this study (Table 13), though there is significant difference between SDM and treatment (Table 15 & Figure 7g) along with RDM and treatment (Table 16 & Figure 7h). A possible explanation for this is the higher initial nutrient content in the higher treatments, encouraging a faster growth response in the first growing season. Whether the growth response would have persisted past the first growing season is undetermined.

Correlations can be found between the RDM and diameter, which then translated to the seedling's survivability (Mullin & Christl 1981) and that the larger the root system the better the seedling will perform. These finding prove similar to results calculated in this study (Figure 10). It is also noted that while this is a viable predictor of seedling quality, variables such as height, diameter, and stem weight are a better fit as predictors (Thompson 1985). RDM did show significance in comparison to treatment level, but only between P0 and P4 (Table 16 & Figure 7h). All other comparisons proved not significant and had no correlation between treatment level and RDM. Since there was no statistical difference among the majority of treatments, root surface area was not a

mechanism that affected nutrient uptake or a major influencing factor in foliar nutrient content.

It is recommended that when considering the RS ratio, the effect of size, parameters such as height, must be considered due to the ratio changing with the seedling size (Carlson & Preisig 1981, Thompson 1985). When comparing the two, trends show a slight trend decrease in productivity as treatment dose increased (Figure 11) though there was no real significance found between RS ratio and treatment (Table 13). Excess soil P increased plant growth between both the root and shoot dry weight in higher treatments (Table 12) as well as P uptake by the plant tissue in all treatments (Table 24 & Figures 7m & 12). Within both the SDM and RDM there was an increase in dry biomass yield (Table 12), though RS ratio was not influenced by excess soil P to any significant extent.

Table 16. Tukey post-hoc test probability for P concentration to affect shoot dry matter treatment interaction.

| **Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: SDM** | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.789 | 0.137 | 0.002 | <.0001 | 0.004 |
| **(P1) 250** | 0.789 | | 0.811 | 0.052 | 0.000 | 0.111 |
| **(P2) 500** | 0.137 | 0.811 | | 0.524 | 0.015 | 0.735 |
| **(P3) 750** | 0.002 | 0.052 | 0.524 | | 0.519 | 0.999 |
| **(P4) 1000** | <.0001 | 0.000 | 0.015 | 0.519 | | 0.322 |
| **(P5) 1250** | 0.004 | 0.111 | 0.735 | 0.999 | 0.322 | |

Table 17. Tukey post-hoc test probability for P concentration to affect root dry matter treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: RDM | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.999 | 0.864 | 0.601 | 0.031 | 0.245 |
| **(P1) 250** | 0.999 | | 0.970 | 0.812 | 0.075 | 0.432 |
| **(P2) 500** | 0.864 | 0.970 | | 0.997 | 0.339 | 0.880 |
| **(P3) 750** | 0.601 | 0.812 | 0.997 | | 0.623 | 0.988 |
| **(P4) 1000** | 0.031 | 0.075 | 0.339 | 0.623 | | 0.934 |
| **(P5) 1250** | 0.245 | 0.432 | 0.880 | 0.988 | 0.934 | |

Table 18. Tukey post-hoc test probability for P concentration to affect root-to-shoot ratio treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: RS ratio | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.910 | 0.747 | 0.297 | 0.373 | 0.825 |
| **(P1) 250** | 0.910 | | 0.999 | 0.877 | 0.928 | 1.000 |
| **(P2) 500** | 0.747 | 0.999 | | 0.973 | 0.990 | 1.000 |
| **(P3) 750** | 0.297 | 0.877 | 0.973 | | 1.000 | 0.944 |
| **(P4) 1000** | 0.373 | 0.928 | 0.990 | 1.000 | | 0.973 |
| **(P5) 1250** | 0.825 | 1.000 | 1.000 | 0.944 | 0.973 | |

## Sturdiness Quotient (SQ) and Dickson Quality Index (DQI)

Seedling quality determinates like SQ were calculated at the beginning of the prior to seedling planting and following the first growing season. Determinates like DQI were calculated following destructive sampling of seedlings. Table 12 shows the mean SQ and DQI by treatment following the first growing season.

Final mean SQ over one growing season for six treatments was not significant ($p > 0.05$) for treatment effect (Table 18). Final SQ was highest in P3 and lowest in P0 (Table 12), means ranging from 4.77 to 5.47. Mean DQI over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 18). Mean DQI was highest in P4 and lowest in P0 (Table 12), means ranging from 5.21 to 7.53.

Adjustments for multiple comparison from the Tukey post-hoc test was performed in tandem with a One-Way ANOVA and reported that the relationship of DQI between the following treatments have strong relations, or hold significance: P0 v. P4 ($p = 0.019$) and P1 v. P4 ($p = 0.046$) (Table 20 & Figure 7l). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 29a-c & 30a-c).

SQ is a determinate of seedling stockiness or lean nature. It is closely correlated to RCD and the higher the calculated variable the sturdier the seedling (Thompson 1985). SQ was found to have no significance between treatments, meaning there was no

statistical difference in seedlings stem vigor. SQ was not affected by variable P application.

DQI was calculated using Eq. 1 and is the evaluation of multiple morphological parameters on a seedling to determine field performance. It was initially developed by Dickson et al. 1960 as a means of evaluating the performance of white pine and white spruce seedlings. The higher the calculated variable, the better the seedling will perform. Results for this study noted that there were only significant differences between P0, P1 and P4 (Table 20). According to the DQI, P4 had the highest field performance during the first growing season, and that high treatment dose will increase seedling performance for the first growing season. It should be mentioned that higher treatments also showed signs of possible nutrient deficiency during the later month of the first growing season. Results may have differed during a longer study timeframe, and could also be inconclusive due to the contrasting original species (white pine and white spruce) this index was evaluated on.

Table 19. Sturdiness quotient and Dickson quality index significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| SQ | Treatment | 0.793 | 0.159 | 0.35 | 0.879 |
| DQI | Treatment | 22.725 | 4.545 | 3.01 | 0.021 |

Table 20. Tukey post-hoc test probability for P concentration to affect sturdiness quotient treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: SQ | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 1.000 | 0.994 | 0.981 | 1.000 | 1.000 |
| **(P1) 250** | 1.000 | | 0.999 | 0.945 | 1.000 | 1.000 |
| **(P2) 500** | 0.994 | 0.999 | | 0.834 | 1.000 | 0.999 |
| **(P3) 750** | 0.981 | 0.945 | 0.834 | | 0.935 | 0.948 |
| **(P4) 1000** | 1.000 | 1.000 | 1.000 | 0.935 | | 1.000 |
| **(P5) 1250** | 1.000 | 1.000 | 0.999 | 0.948 | 1.000 | |

Table 21. Tukey post-hoc test probability for P concentration to affect Dickson quality index treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: DQI | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.999 | 0.506 | 0.647 | 0.019 | 0.290 |
| **(P1) 250** | 0.999 | | 0.721 | 0.840 | 0.046 | 0.482 |
| **(P2) 500** | 0.506 | 0.721 | | 1.000 | 0.598 | 0.999 |
| **(P3) 750** | 0.647 | 0.840 | 1.000 | | 0.458 | 0.990 |
| **(P4) 1000** | 0.019 | 0.046 | 0.598 | 0.458 | | 0.822 |
| **(P5) 1250** | 0.290 | 0.482 | 0.999 | 0.990 | 0.822 | |

## Ectomycorrhizal Root Tip Development

Plant growth attributes such as EMRT were calculated following the destructive sampling of the seedling. Table 21 shows the mean EMRT count of all seedlings per treatment following the first growing season.

Table 22. Mean ectomycorrhizal root tip count per treatment after the first growing season.

| **Treatment** (mg P/kg media) | **Parameters** Mean EMRT count (# / seedling) |
|---|---|
| **(P0) Control** | 17291 |
| **(P1) 250** | 20586 |
| **(P2) 500** | 17799 |
| **(P3) 750** | 20793 |
| **(P4) 1000** | 21940 |
| **(P5) 1250** | 17426 |

The EMRT count following one growing season for six treatments was not significant ($p > 0.05$) for treatment effect (Figure 22). The EMRT count was highest in P4 and lowest in P0 (Table 21), means ranging from 17,291 to 21,940 mean estimated EMRT counts. Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figure 31a-c).

Trends between EMRT count and RDM are positive and significant ($p < 0.0001$) (Figure 12), though it is not significant enough to be influenced by treatment dosage (Table 22). Possible influence could have come from the leaching of phosphorus to the

bottom of the pot, causing the growth of EMRT in preferential areas with low stress nutrient concentrations rather than root lengths surrounded by high P concentrations. As it stands, in the boundaries of this study EMRT count is not influenced by variable P application.

Table 23. Ectomycorrhizal root tip count significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| EMRT count | Treatment | 165226658.3 | 33045331.7 | 0.66 | 0.655 |

Table 24. Tukey post-hoc test probability for P concentration to affect estimated ectomycorrhizal root tip count treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: EMRT count | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 1.000 | 0.308 | 0.909 | 0.990 | 0.962 |
| **(P1) 250** | 1.000 | | 0.294 | 0.898 | 0.987 | 0.956 |
| **(P2) 500** | 0.308 | 0.294 | | 0.887 | 0.676 | 0.796 |
| **(P3) 750** | 0.909 | 0.898 | 0.887 | | 0.999 | 1.000 |
| **(P4) 1000** | 0.990 | 0.987 | 0.676 | 0.999 | | 1.000 |
| **(P5) 1250** | 0.962 | 0.956 | 0.796 | 1.000 | 1.000 | |

## Nutrient Analysis and Deficiency Symptoms

Plant nutrient uptake content of P, Zn, and Fe was determined following the destructive sampling of the seedlings. Table 24 shows the mean P, Zn, Fe, and other base nutrient concentrations by treatment. All other base nutrients accumulated in the plant leaf tissue were at acceptable levels for development (Table 24). Elevated calcium by treatment in the foliar nutrient analysis can be explained by the product makeup of P fertilizer used. Some P applied to treatments was expected to drain from seedling pots with watering, though efforts were put in place to minimize drainage. Triple superphosphate is soluble phosphate fertilizer that also contains on average 14% calcium and comes in the form of calcium dihydrogen phosphate (Zimdahl 2015). Elevated Mn was found to be significant in relation to increasing treatment level and increased foliar P uptake. An explanation would be that triple superphosphate frequently stimulates an increased uptake of Mn with it into the plant. The increased uptake of Mn as treatment level increased did not have any major repercussions in terms of seedling health. A *P. taeda* L. standard was sent to the SFASU Soil Testing Laboratory along with the foliage samples for quality control. Results matched the analysis data sheet for quality control.

### Phosphorus

P uptake over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 25). P was highest in P5 and lowest in P0 (Table 24), means ranging from 1679 mg $kg^{-1}$ to 11819 mg $kg^{-1}$.

Adjustment for pairwise comparison was performed in tandem with a linear regression and reported the interactions of P uptake and treatment dose. The following treatment pairs were found to have strong relation, or hold significance: P0 v. P1 ($p = <0.0001$), P0 v. P2 ($p = <0.0001$), P0 v. P3 ($p = <0.0001$), P0 v. P4 ($p = <0.0001$), P0 v. P5 ($p = <0.0001$), P1 v. P4 ($p = 0.0002$), P1 v. P5 ($p = <0.0001$), P2 v. P4 ($p = 0.0081$), P2 v. P5 ($p = <0.0001$), P3 v. P5 ($p = <0.0001$), and P4 v. P5 ($p = 0.0023$) (Table 26 & Figure 7m). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figure 32a-c).

Zinc and Iron

Zn uptake over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 25). Zn was highest in P4 and lowest in P0 (Table 24), means ranging from 35 mg $kg^{-1}$ to 70 mg $kg^{-1}$. Fe uptake over one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 25). Fe was highest in P1 and lowest in P5 (Table 24), means ranging from 58 mg $kg^{-1}$ to 96 mg $kg^{-1}$; possibly indicating a competitive interaction between P and Fe uptake. Deficiency symptoms such as chlorotic plant tissue and tip necrosis were recorded throughout the first growing season (Table 29) and were found to have significant ($p < 0.05$) relations between the presence of chlorosis, necrosis, and treatment effect (Table 30).

Adjustment for pairwise comparison was performed in tandem with a linear regression and reported the interactions of Zn uptake and treatment dose. The following

treatment pairs were found to have strong relation, or hold significance: P0 v. P1 ($p = 0.0127$), P0 v. P2 ($p = 0.0001$), P0 v. P3 ($p = 0.0002$), P0 v. P4 ($p = <0.0001$), and P0 v. P5 ($p = 0.0029$) (Table 28 & Figure 7o). Adjustment for pairwise comparison was performed in tandem with a linear regression and reported the interactions of Fe uptake and treatment dose. The following treatment pairs were found to have a strong relationship, or hold significance: P1 v. P5 ($p = 0.0341$) (Table 27 & Figure 7n). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 33a-c, 34a-c, 35a-c & 36a-c).

Table 25. Mean foliage nutrient concentration for macro- and micronutrients (mg $kg^{-1}$) per treatment at the end of the first growing season.

| **Treatment** | **Macronutrients** | | | | | **Micronutrients** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (mg P/kg media) | P | K | S | Ca | Mg | B | Fe | Mn | Zn | Cu | Na |
| | ---------- mg $kg^{-1}$ ---------- | | | | | ---------- mg $kg^{-1}$ ---------- | | | | | |
| **(P0) Control** | 1679$^{d}$ | 12327 | 1251 | 3048 | 1186 | 23 | 79$^{ab}$ | 298 | 35$^{b}$ | 5 | 382 |
| **(P1) 250** | 6259$^{c}$ | 15365 | 1460 | 4888 | 1748 | 32 | 96$^{a}$ | 394 | 59$^{a}$ | 5 | 313 |
| **(P2) 500** | 6985$^{c}$ | 14240 | 1250 | 5227 | 1975 | 35 | 70$^{ab}$ | 525 | 69$^{a}$ | 4 | 495 |
| **(P3) 750** | 7921$^{bc}$ | 13790 | 1265 | 5087 | 2046 | 35 | 62$^{ab}$ | 591 | 68$^{a}$ | 3 | 422 |
| **(P4) 1000** | 9266$^{b}$ | 13555 | 1232 | 5129 | 2102 | 32 | 72$^{ab}$ | 761 | 70$^{a}$ | 3 | 600 |
| **(P5) 1250** | 11819$^{a}$ | 15729 | 1346 | 5919 | 2241 | 37 | 58$^{b}$ | 844 | 62$^{a}$ | 3 | 447 |

Means in a column followed by the same letter had no significant difference at the 0.05 level. Mean comparisons were determined by the Tukey post hoc test.

In this study Fe and Zn were analyzed for influence during phosphorus uptake, as well as any symptoms of deficiency. Physical defects noted throughout the course of this study include the previously mentioned wilting needles and branches (Figures 43-48), in addition to chlorosis of plant tissue and tip necrosis (Table 30). Deficiency symptoms such as these were more numerous the higher the treatment level (Table 29). It is a repeated result in horticulture research involving plants such as maize, clover, pinto bean, and wheat that excessive P soil content can cause Zn and Fe deficiencies (Millikan 1963, Watanabe et al. 1965, Stuckenholtz et al. 1966, Shahzad et al. 2014, Ova et al. 2015, Shi et al. 2018). This is typically presented in physical traits such as chlorotic tissue pigment or tip necrosis (Provin & Pitt 2008, Kuldeep 2009).

Related research notes that even through the plant tissue may house an absolute available iron-content, the P:Fe ratio will most likely be the determinate of whether the plant tissue will appear chlorotic or healthy (De Kock & Wallace 1965). Some research even considers Fe-deficiency to be equivalent to P:Fe dilution since plants exposed to excess P are expected to experience chlorosis, which can be amended with Fe (De Kock & Wallace 1965, Shi et al. 2018, Xie et al. 2019). These findings align with results presented in this study. Mean Fe uptake was found to be significant (Table 25), but only between P1 and P5 (Table 27 & Figure 7n). P uptake in plant tissue increases greatly as treatment level increases (Table 25). Fe to P ratios between P1 and P6 had a percent reduction of 68%, showing trends of Fe dilution as a result of raised foliar P concentrations (Table 24). Figures 13 and 14 and demonstrate visual trends where P has

a positive regression and Fe has a negative regression as treatment dosage increases. It is possible that excess soil P influenced Fe foliar presence through dilution effect or possible P:Fe competitive interaction (Figure 16) and slight trending decrease in Fe as treatment rate increased (Figure 14). The ratio of P to Fe increased with the treatment level, in addition to an increased appearance of possible deficiency symptoms such as plant tissue chlorosis and tip necrosis (Table 29).

Zn uptake increased with treatment P dosage (Table 25 & Figure 15) where relations between the control and all treatment levels showed significance (Table 28). Zn to P ratios between P1 and P6 had a percent reduction of 42%, showing trends of Zn dilution as a result of raised foliar P concentrations (Table 24). Figures 13 and 15 and demonstrate visual trends where P has a positive regression and Zn has a positive regression as treatment dosage increases. Variation from this regression line follows the treatment differences between P1 and P5, where mean Zn foliar concentration decreases and drop out of the positive trend. It is possible that excess soil P was a factor affecting Zn foliar presence in both dilution effect (Figure 17 & 18) in P1, P2, P3, P4, and P5 as well as nutrient uptake mechanisms in P5. Deficiency symptoms can be explained through a dilution effect rather than reduced Zn uptake into the seedling (Soltanghesi et al. 2014), though results vary (Xie et al. 2019). The potting media supplied to the seedling had limited nutrients available so with the increased growth caused by the P application, Zn became diluted within the tissue leading to potential Zn deficiency in the higher treatments where the P:Zn ratios were disproportionally larger (Figure 18).

Similar relations were accumulated in literature by Soltanghesi et al. 2014 while studying the relation of P and Fe within various plant tissues. Results are variable, as some research notes that P uptake will decrease the total uptake of Zn in plant tissue (Stukenholtz et al. 1966, Xie et al. 2019), while others P will either have no effect on Zn uptake (Millikan 1963), or have increased Zn uptake (Watanabe et al. 1965). Notably all mentioned research has mention of Zn deficiency present.

Excess nutrient content of P can lead to the deficiency of another nutrient, such as Fe or Zn, through dilution of the plant nutrient balance or through nutrient competition effects. In the bound of this study, P has the potential to dilute Fe and Zn within *P. taeda* L. to an extent that promotes deficiency symptoms such as chlorosis or tip necrosis. Results provided during an additional growing season could show the prolonged morphological effects of these deficiencies on *P. taeda* L.

Table 26. Phosphorus, Iron, and Zinc significance of interaction by treatment after one growing season utilizing Type III SS.

| Parameter | Source | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| P | Treatment | 459606737.200 | 91921347.400 | 59.84 | <.0001 |
| Fe | Treatment | 7467.354 | 1493.471 | 2.53 | 0.043 |
| Zn | Treatment | 6978.604 | 1395.721 | 7.66 | <.0001 |

Table 27. Tukey post-hoc test probability for P soil concentration to influence P uptake treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: P | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |
| **(P1) 250** | <.0001 | | 0.847 | 0.101 | 0.000 | <.0001 |
| **(P2) 500** | <.0001 | 0.847 | | 0.661 | 0.008 | <.0001 |
| **(P3) 750** | <.0001 | 0.101 | 0.661 | | 0.272 | <.0001 |
| **(P4) 1000** | <.0001 | 0.000 | 0.008 | 0.272 | | 0.002 |
| **(P5) 1250** | <.0001 | <.0001 | <.0001 | <.0001 | 0.002 | |

Table 28. Tukey post-hoc test probability for P soil concentration to influence Fe uptake treatment interaction.

| Probability for Treatment Effect<br>Pr > \|t\| for H0<br>Dependent Variable: Fe | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.726 | 0.967 | 0.714 | 0.994 | 0.514 |
| **(P1) 250** | 0.726 | | 0.262 | 0.073 | 0.389 | 0.034 |
| **(P2) 500** | 0.967 | 0.262 | | 0.988 | 1.000 | 0.932 |
| **(P3) 750** | 0.714 | 0.073 | 0.988 | | 0.950 | 1.000 |
| **(P4) 1000** | 0.994 | 0.389 | 1.000 | 0.950 | | 0.837 |
| **(P5) 1250** | 0.514 | 0.034 | 0.932 | 1.000 | 0.837 | |

Table 29. Tukey post-hoc test probability for P soil concentration to influence Zn uptake treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Zn | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 0.013 | 0.000 | 0.000 | <.0001 | 0.003 |
| **(P1) 250** | 0.013 | | 0.655 | 0.733 | 0.584 | 0.995 |
| **(P2) 500** | 0.000 | 0.655 | | 1.000 | 1.000 | 0.915 |
| **(P3) 750** | 0.000 | 0.733 | 1.000 | | 1.000 | 0.952 |
| **(P4) 1000** | <.0001 | 0.584 | 1.000 | 1.000 | | 0.874 |
| **(P5) 1250** | 0.003 | 0.995 | 0.915 | 0.952 | 0.874 | |

Table 30. Observed presence of both plant tissue chlorosis and tip necrosis per treatment at the end of the first growing season.

| **Treatment** | **Chlorosis** | | **Necrosis** | |
|---|---|---|---|---|
| (mg P/kg media) | Present | Not Present | Present | Not Present |
| **(P0) Control** | $0^{b}$ | 8 | $0^{c}$ | 8 |
| **(P1) 250** | $0^{b}$ | 8 | $0^{c}$ | 8 |
| **(P2) 500** | $5^{a}$ | 3 | $1^{bc}$ | 7 |
| **(P3) 750** | $6^{a}$ | 2 | $2^{bc}$ | 6 |
| **(P4) 1000** | $8^{a}$ | 0 | $4^{b}$ | 4 |
| **(P5) 1250** | $8^{a}$ | 0 | $8^{a}$ | 0 |

Means in a column followed by the same letter had no significant difference at the 0.05 level. Mean comparisons were determined by the Tukey post hoc test.

Table 31. Plant tissue chlorosis and tip necrosis significance of interaction by treatment after one growing season utilizing Type III SS.

| **Parameter** | **Source** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|
| Chlorosis | Treatment | 8.438 | 1.688 | 21.00 | <0.0001 |
| Necrosis | Treatment | 5.938 | 1.188 | 11.40 | <0.0001 |

Table 32. Tukey post-hoc test probability for P concentration to affect plant tissue chlorosis treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Chlorosis | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 1.000 | 0.001 | <.0001 | <.0001 | <.0001 |
| **(P1) 250** | 1.000 | | 0.001 | <.0001 | <.0001 | <.0001 |
| **(P2) 500** | 0.001 | 0.001 | | 0.949 | 0.109 | 0.109 |
| **(P3) 750** | <.0001 | <.0001 | 0.949 | | 0.499 | 0.499 |
| **(P4) 1000** | <.0001 | <.0001 | 0.109 | 0.499 | | 1.000 |
| **(P5) 1250** | <.0001 | <.0001 | 0.109 | 0.499 | 1.000 | |

Table 33. Tukey post-hoc test probability for P concentration to affect plant tissue necrosis treatment interaction.

| Probability for Treatment Effect Pr > \|t\| for H0 Dependent Variable: Necrosis | | | | | | |
|---|---|---|---|---|---|---|
| **Treatment** | **Control** | **250** | **500** | **750** | **1000** | **1250** |
| **(P0) Control** | | 1.000 | 0.970 | 0.635 | 0.038 | <.0001 |
| **(P1) 250** | 1.000 | | 0.970 | 0.635 | 0.038 | <.0001 |
| **(P2) 500** | 0.970 | 0.970 | | 0.970 | 0.208 | <.0001 |
| **(P3) 750** | 0.635 | 0.635 | 0.970 | | 0.635 | 0.000 |
| **(P4) 1000** | 0.038 | 0.038 | 0.208 | 0.635 | | 0.038 |
| **(P5) 1250** | <.0001 | <.0001 | <.0001 | 0.000 | 0.038 | |

## Munsell Color Analysis With and Without Cuticle

Often seedling color is viewed as a subjective measure of quality. Value to color is given through a complete observation, such as seedlings with yellow, or brown coloring will have less vigor and not as healthy as ones with deep green coloring (Thompson 1985). This study attempted to branch further, utilizing the Munsell color system for plant tissues as a tool for evaluating plant health in the 3-dimensional color space. Munsell color for plant tissue was recorded after initial planting, before harvest for living color, and subsequent drying and grinding to break up the waxy cuticle.

Starting color for all seedlings before P application was 5GY 5/6, a healthy green color. Munsell color with cuticle intact after one growing season for six treatments was significant ($p < 0.05$) for treatment effect (Table 33) and had variable change compared to initial color. Of the three axes combined the one with the most significance is the X axis, a Cartesian coordinate transformation of the Munsell Hue and Chroma. Excess P in moist living *P. taeda* L. needles caused the highest shift within 3-dimensional Euclidean space between x-axis lines 5.71 and 7.90 (Figure 39). This is validated by stepwise regression results including the x-axis in the final created model (Table 34). Model assumptions follow fit criteria of normal distribution, equal variance, and independence, though lacking due to small sample size (Figures 37a-c & 38a-c).

Munsell color of the dried ground needles after one growing season for six treatments, was significant ($p < 0.05$) for treatment effect (Table 33) and held more

variability within the 3-dimensional Euclidean space than both initial color and living color. All three axes held significance within the 3-dimensional color space, ranging further than the living color (Table 35). High P treatments in dried and ground *P. taeda* L. needles caused the highest shift in the 3-dimensional Euclidean space between y-axis lines 1.24 and 2.72 (Figure 40). These results are validated by stepwise regression results including all three axes in the final model, x- and y-axes as transformations of the Munsell Hue and Chroma to Cartesian coordinates, and the z-axis as the Munsell Value (Table 35).

Color shift within the dried samples had a noticeable visible shift as treatment increased (Figure 5). When comparing samples with and without cuticle, non-cuticle samples had more variation between Munsell color charts and in the 3-dimensional color space. As treatment P dose increases, the less saturated the plant tissue is in color and the more likely it is to develop chlorosis or tip necrosis (Table 29). Range of deficiency in living color for *P. taeda* L. this study lies between the transformed coordinates of (5.71, -1.85, 6) and (7.90, -1.25, 6) or 5GY 6/6 and 2.5GY 6/8 in the Munsell color book for plant tissues. Range of deficiency in dried and ground color for *P. taeda* L. in this study lies between the transformed coordinates of (3.56, 1.82, 6) and (5.34, 1.24, 6) or 5Y 6/4 and 2.5Y 6/6 in the Munsell color book for plant tissues.

Living color treatment comparison of P1, the healthiest treatment in terms of color with an average Munsell color of 5GY 6/6, and P5, the least healthy treatment in

terms of color with an average Munsell color of 2.5GY 8/8. These treatments had a more saturated color, positioned across the y-axis from dried and ground samples. Variation is minimal in the 3-dimensional color space as treatments with chlorotic tissue (P5) trend around point (7.90, -1.25, 6) and tissue with healthier 'dark green' color (P1) (Thompson 1985) trends around point (5.70, -1.85, 6) (Figure 41). Dried and ground color treatment comparison of P1, the healthiest treatment in terms of color with an average Munsell color of 5Y 6/4, and P5, the least healthy treatment in terms of color with an average Munsell color of 2.5Y 7/6. Variation between dried color is higher as treatments with chlorotic tissue (P5) trend between points (3.56, 1.81, 6) and (5.43,2.72, 7) (Figure 41). Tissue with healthier color trends around between points (3.56, 1.81, 6) and (4.75, 1.54, 6). All points from these samples sit in the positive quadrant of the color space (Figure 42) where differences between color can be determined through clustering effect. These methods would benefit from a larger sample size to better show trends. A higher range in deficiency color in *P. taeda* L. from excess soil P can be found in dried and ground foliar samples after the waxy cuticle has been broken up.

Expansion on this research should follow up on recommendations made by D' Andrade & Romney (2003), utilizing the Munsell color system to expand color assessment with a common and easily measurable parameter. This can be extended to other factors that can influence color, including site characteristics (i.e., erosion), soil properties, plant species, and plant species divergence.

Table 34. Living and dried Munsell color significance of interaction by treatment after one growing season.

| Parameter | Source | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Living Color | Treatment | 27.491 | 9.164 | 3.58 | 0.021 |
| Ground Color | Treatment | 57.256 | 19.085 | 10.15 | <.0001 |

Table 35. Logistic regression of living Munsell color samples with cuticle intact. Model created with stepwise selection at the end of the first growing season.

| Step | Effect | Number In | Chi-Square | Pr > Chi Sq |
|---|---|---|---|---|
| 1 | X | 1 | 8.737 | 0.003 |

Table 36. Logistic regression of dried and ground Munsell color samples with removed cuticle. Model created with stepwise selection at the end of the first growing season.

| Step | Effect | Number In | Chi-Square | Pr > Chi Sq |
|---|---|---|---|---|
| 1 | Y | 1 | 12.130 | 0.001 |
| 2 | Z | 2 | 9.003 | 0.003 |
| 3 | X | 3 | 3.149 | 0.076 |


Figure 5. Ground sample from each seedling by treatment and replication (first value; (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$) and the replication (the second value).

# CONCLUSION

Multiple significant responses were detected among P treatments after one growing season. Morphological parameters such as height growth, diameter growth, needle and branch length, TDM, SDM, and RDM all increased in their growth productivity as treatment dosage increased. This is most likely a product of opportunistic growth in the presence of excess nutrients. The treatment with the highest yield for growth was found to be the P4. Parameters such as needle and branch length were also affected by nutrient deficiency symptoms such as, needle wilting, plant tissue chlorosis and tip necrosis. All symptoms had a higher frequency rate the higher the treatment P dose.

Seedling quality indices such as RS ratio, SQ, and DQI were found to either hold no significance or have little relation to treatment effect. EMRT count within the parameters of this study was not significant and was not found to be a limiting factor within this study.

Based on seedling foliar nutrient data collected in this study, macronutrients such as P showed high variance in foliar nutrient concentrations by increasing greatly as treatment increases. Micronutrients such as Fe and Zn both held significance between P treatment and P uptake, where Fe concentration decreased and Zn increasing with rising

treatment levels. In this study, excess P concentrations can lead to deficiency of Fe and Zn through a dilution of the nutrient balance in the foliar tissue, or through mechanisms like nutrient competition. All other nutrient in the foliar nutrient data met base nutrient requirements. pH was non-significant and was not a limiting factor within the binds of this study.

Color shift between living color and dried and ground color were both significant for treatment effect. Living color was only significant in the x-axis of the 3-dimensional color space, while dried and ground color was significant in the x-, y-, and z-axis of the 3-dimenstional color space. The higher the P treatment the less saturated the plant tissue color was and the more likely it was to develop deficiency symptoms. Plant tissue color has the potential to be a tool used for plant tissue analysis to separate color and variable plant tissue nutrient deficiencies. A color range for excess soil P for *P. taeda* L. for living and dried and ground color is provided within discussion.

Many parameters within this study could have benefitted from another growing season to help define the result variables. Year and treatment have high chance of being significant within the interactions of growth yield parameters, nutrient uptake, and plant color. Results during this timeframe may vary from results presented within this study.

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**APPENDIX A** – Tables and Figures

Means by Parameter and Treatment

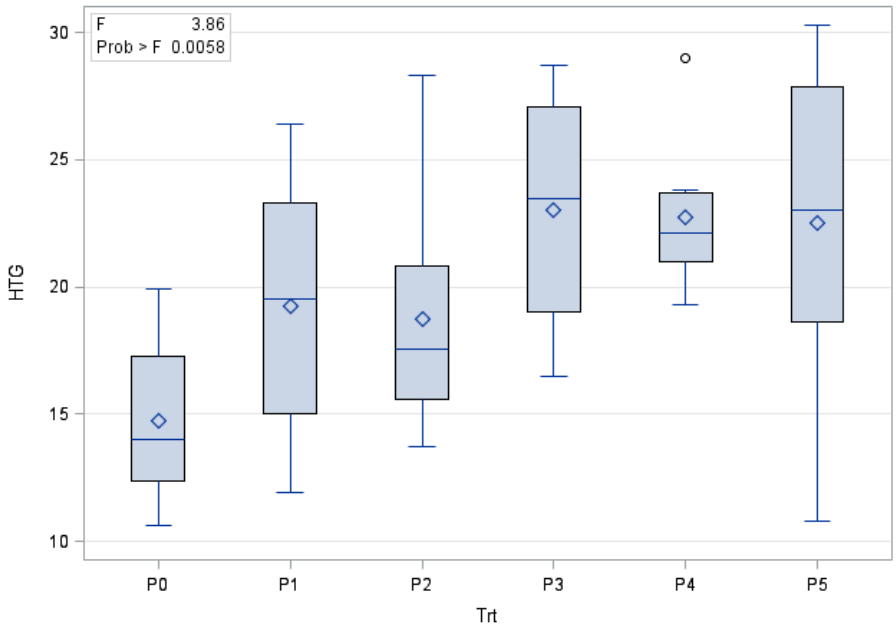


Figure 6.a. Mean height growth (HTG) (cm) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

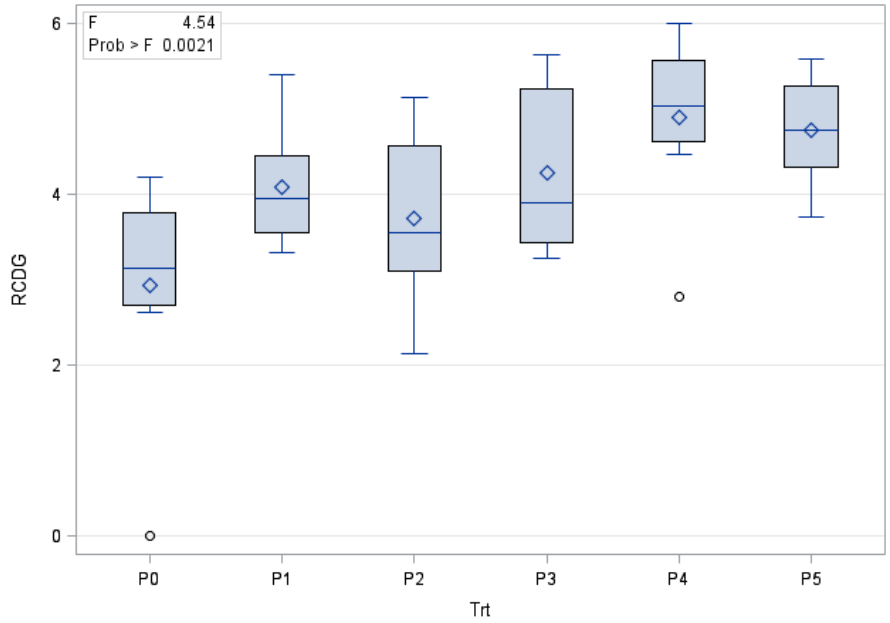


Figure 6.b. Mean diameter growth (RCDG) (mm) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

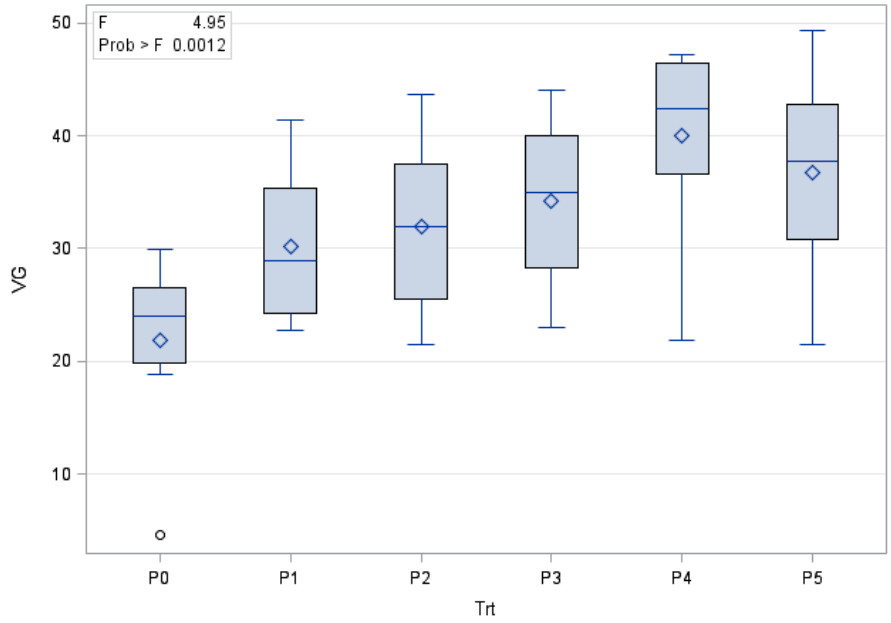


Figure 6. c Mean volume growth (VG) ($cm^3$) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

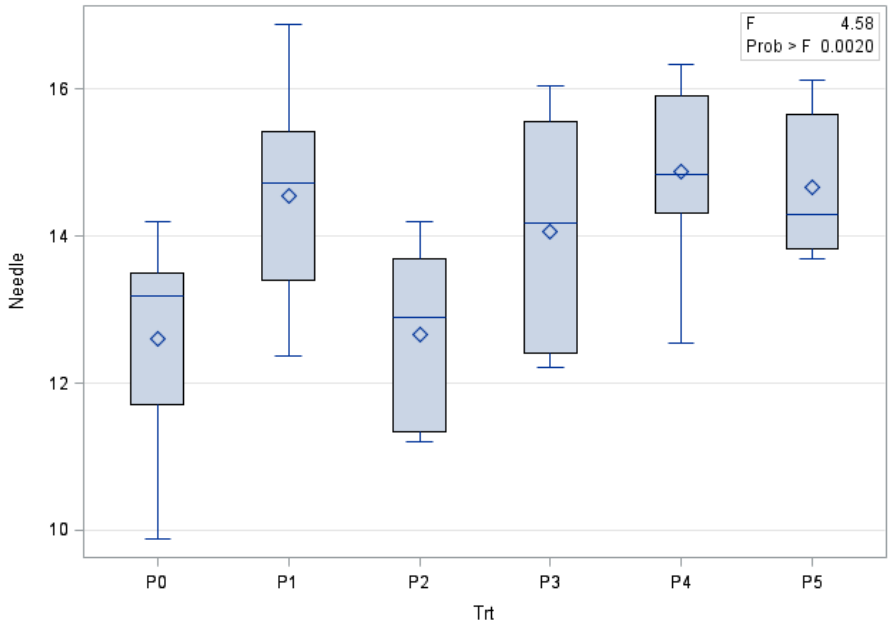


Figure 6.d. Mean needle length (Needle) (cm) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

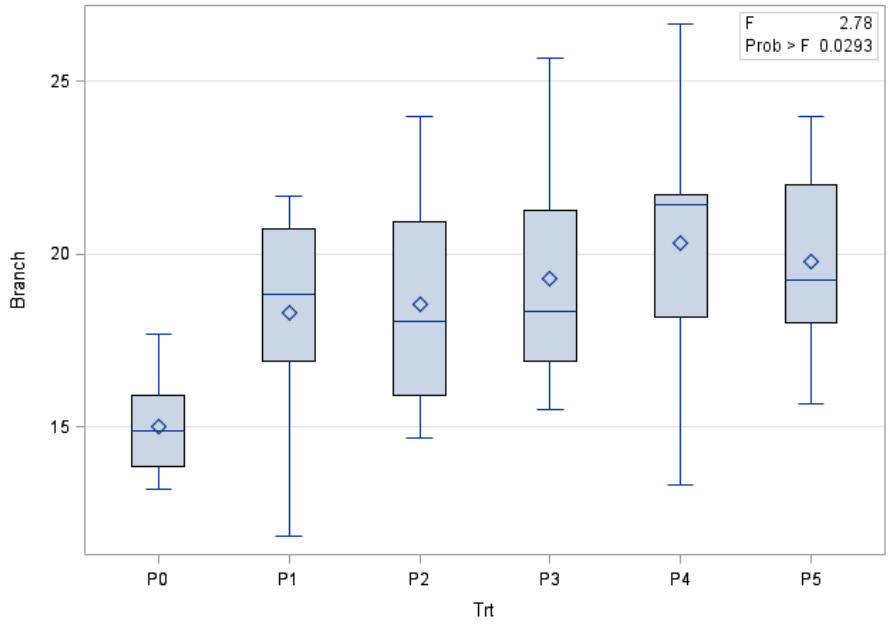


Figure 6.e. Mean branch length (Branch) (cm) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

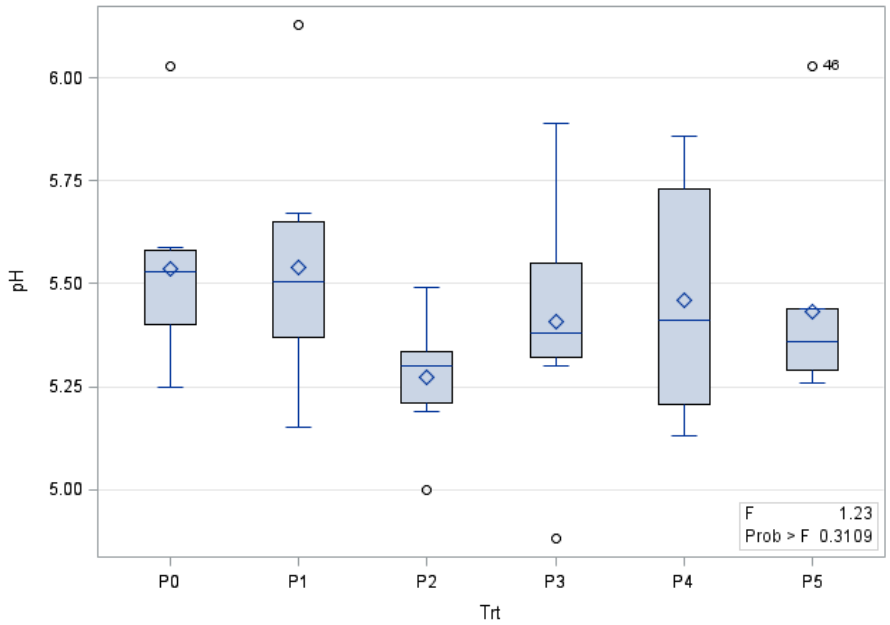


Figure 6.f. Mean pH distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

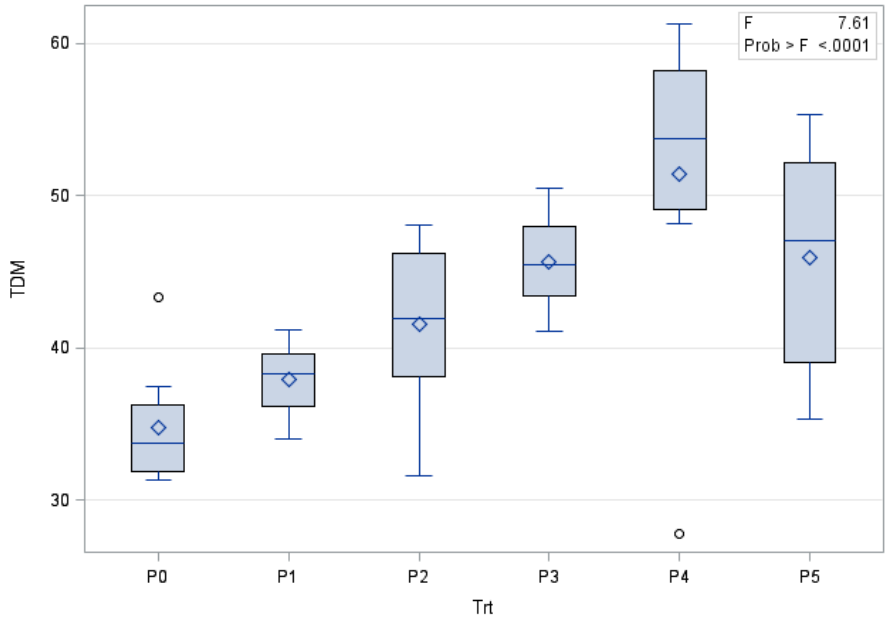


Figure 6.g. Mean total dry matter (TDM) (g) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

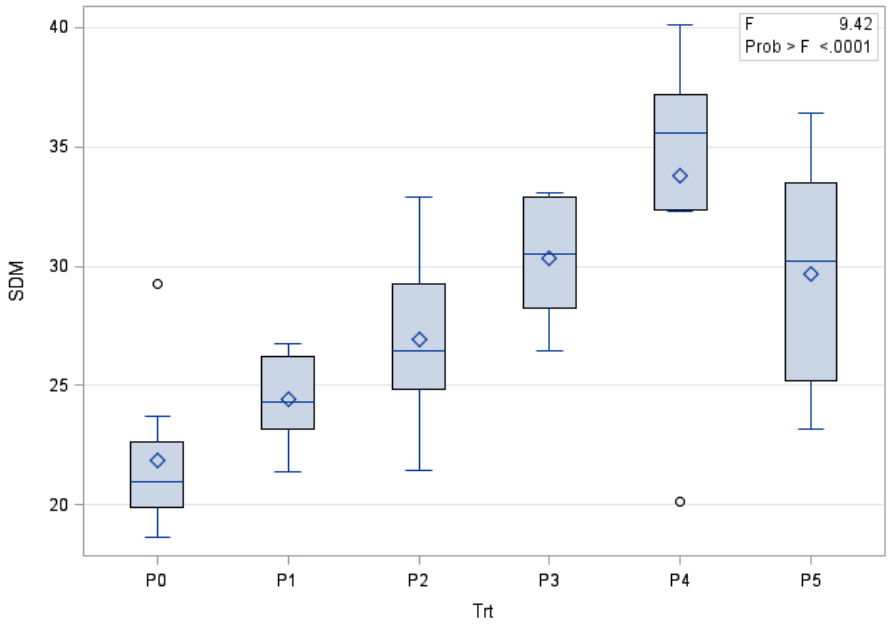


Figure 6.h. Mean shoot dry matter (SDM) (g) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

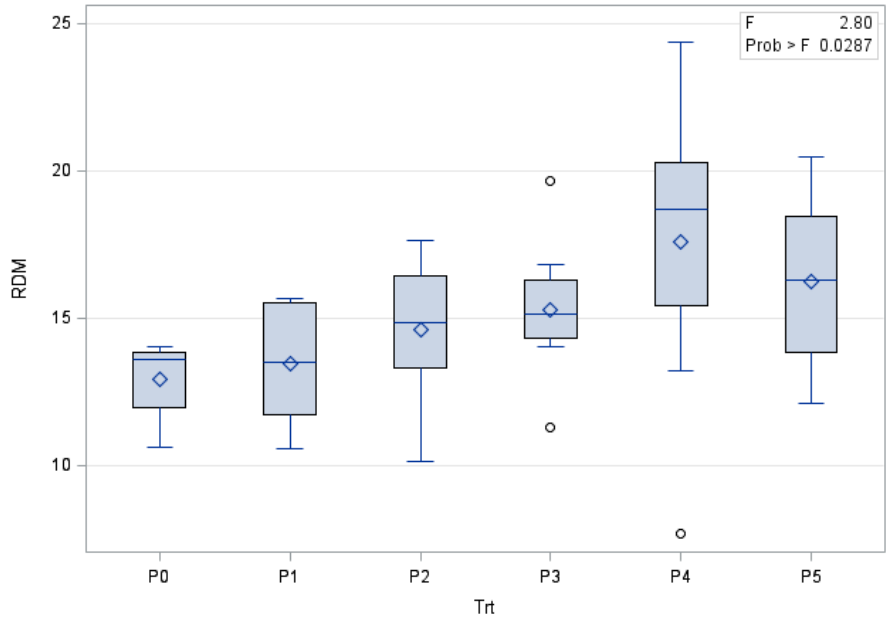


Figure 6.i. Mean root dry matter (RDM) (g) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

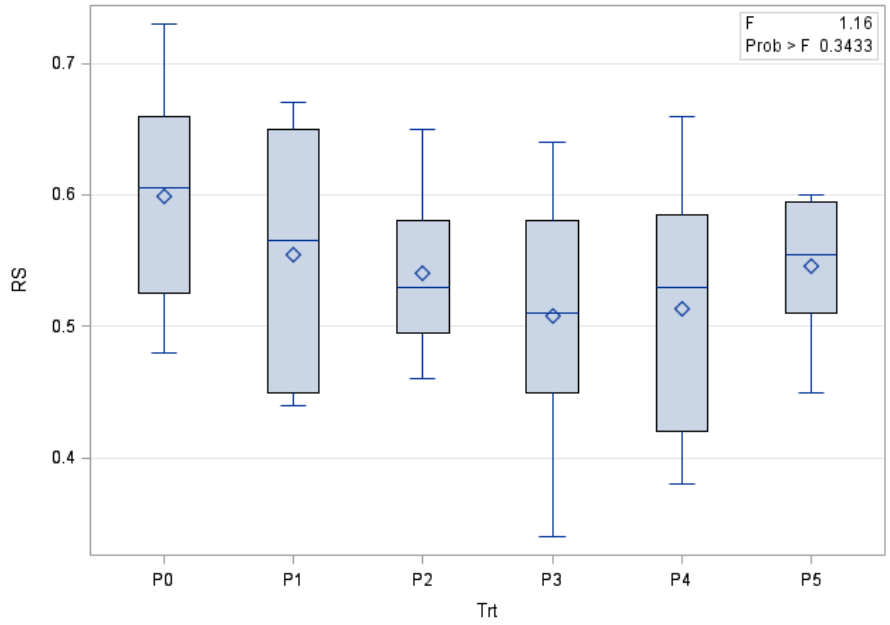


Figure 6.j. Mean root-to-shoot ratio (RS) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

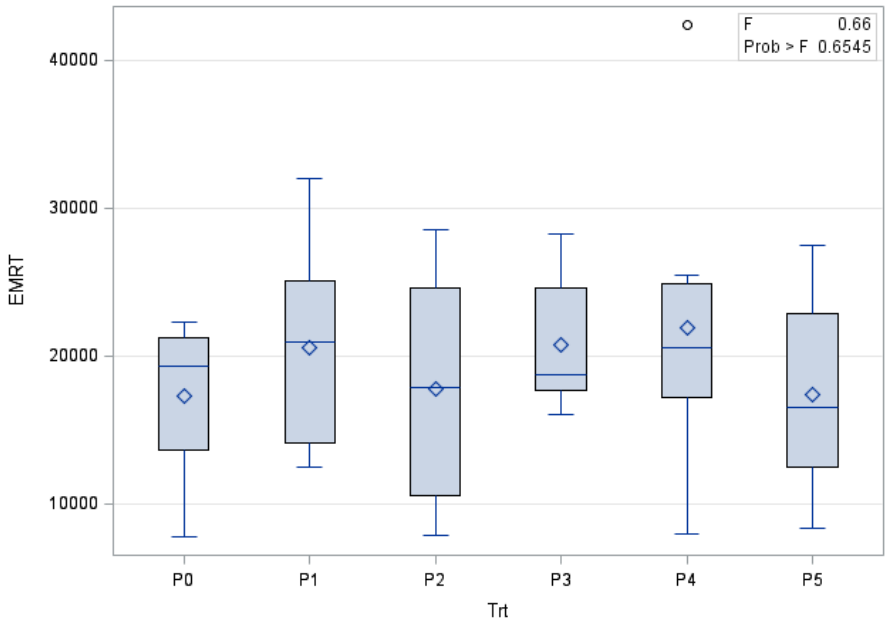


Figure 6 k. Mean ectomycorrhizal root tip count (EMRT) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

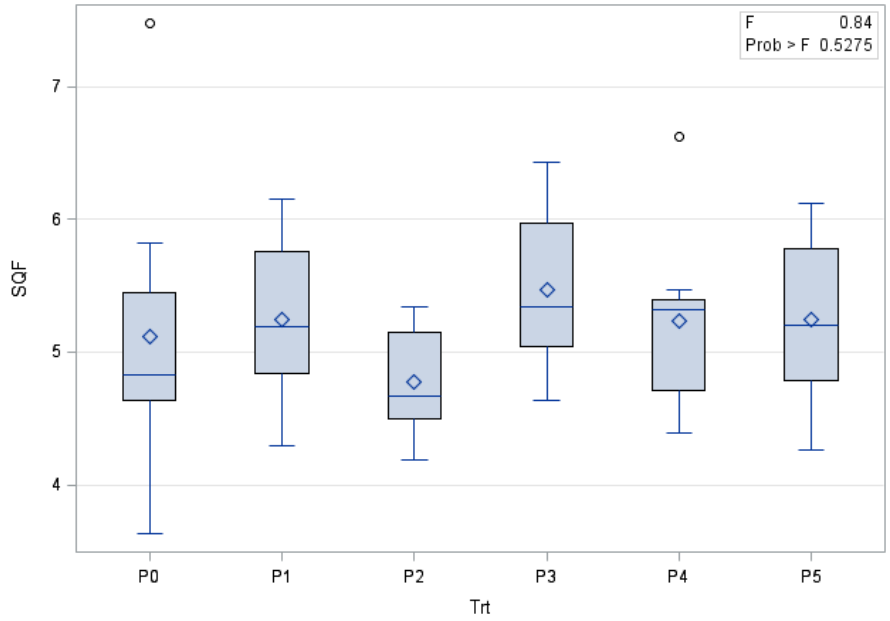


Figure 6.l. Mean sturdiness quotient (SQ) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

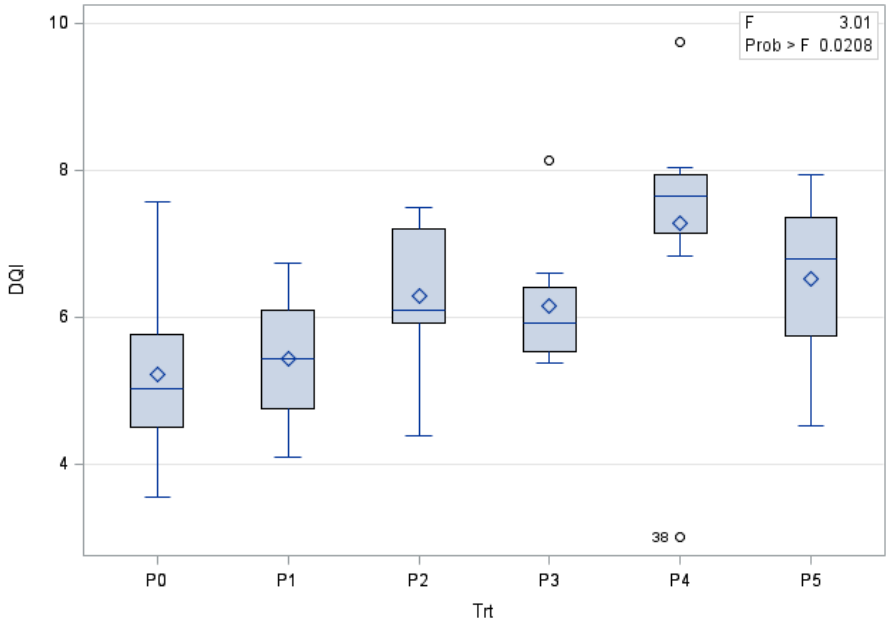


Figure 6.m. Mean Dickson quality index (DQI) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

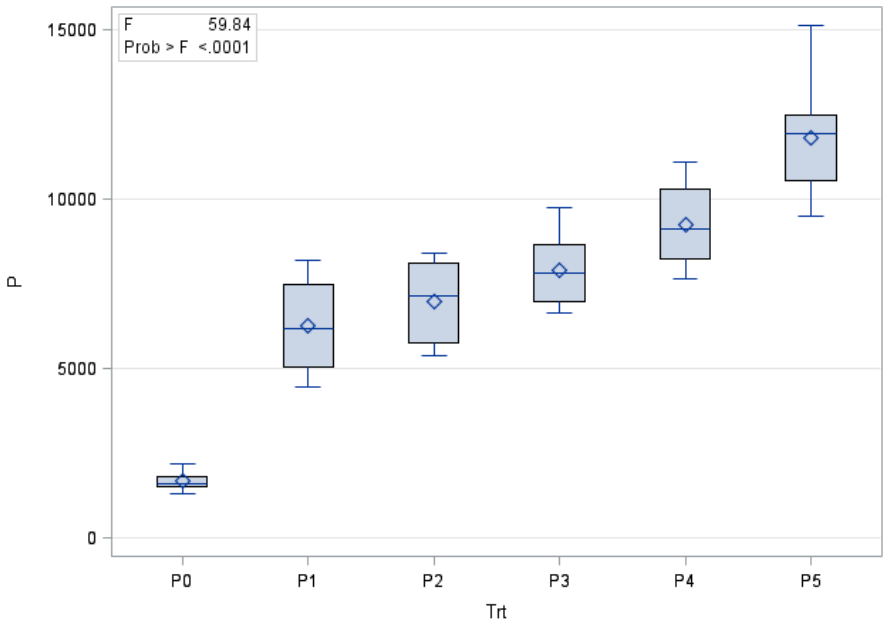


Figure 6.n. Mean phosphorus foliar concentration (P) (mg $kg^{-1}$) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

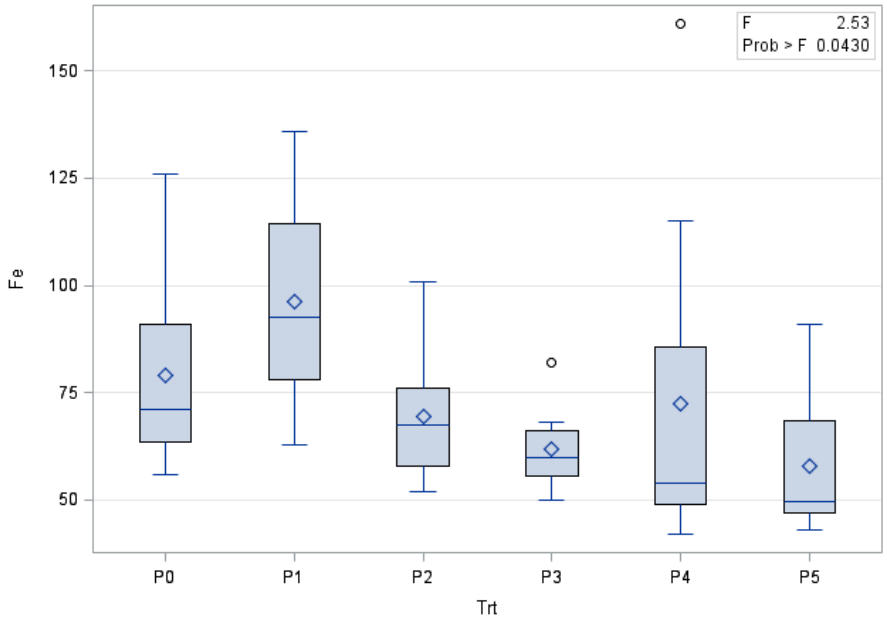


Figure 6.o. Mean iron foliar content (Fe) (mg $kg^{-1}$) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

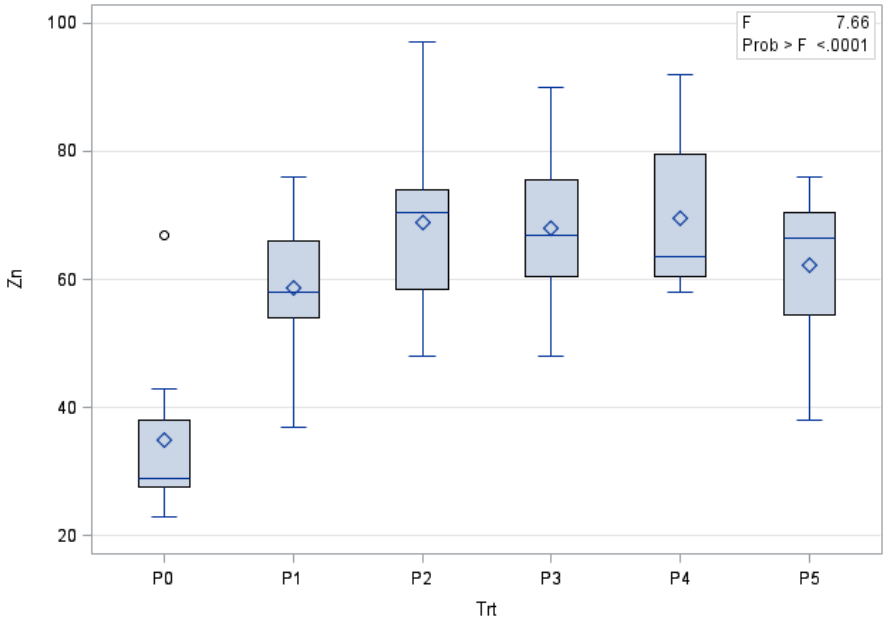


Figure 6.p. Mean zinc foliar content (Zn) (mg $kg^{-1}$) distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

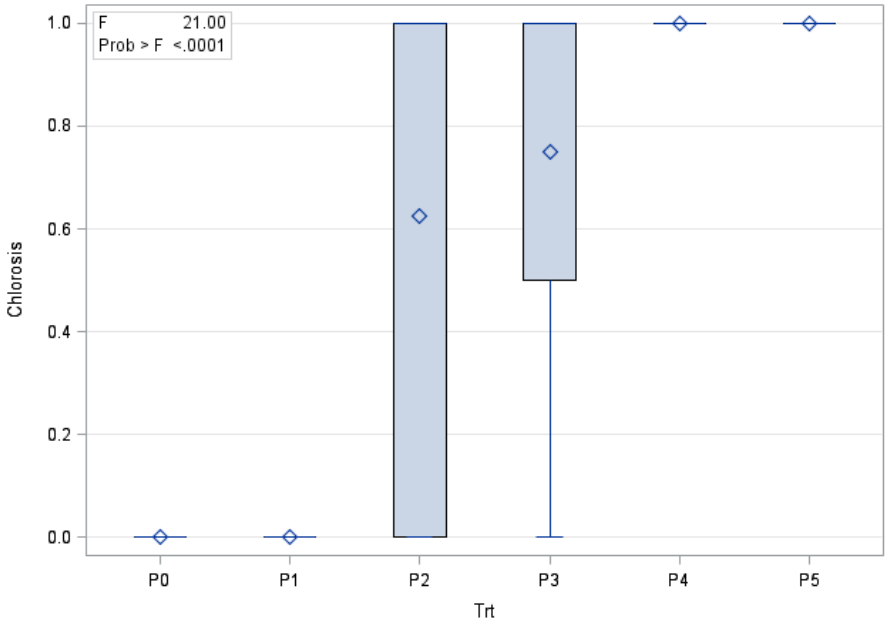


Figure 6.q. Mean chlorotic observation distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

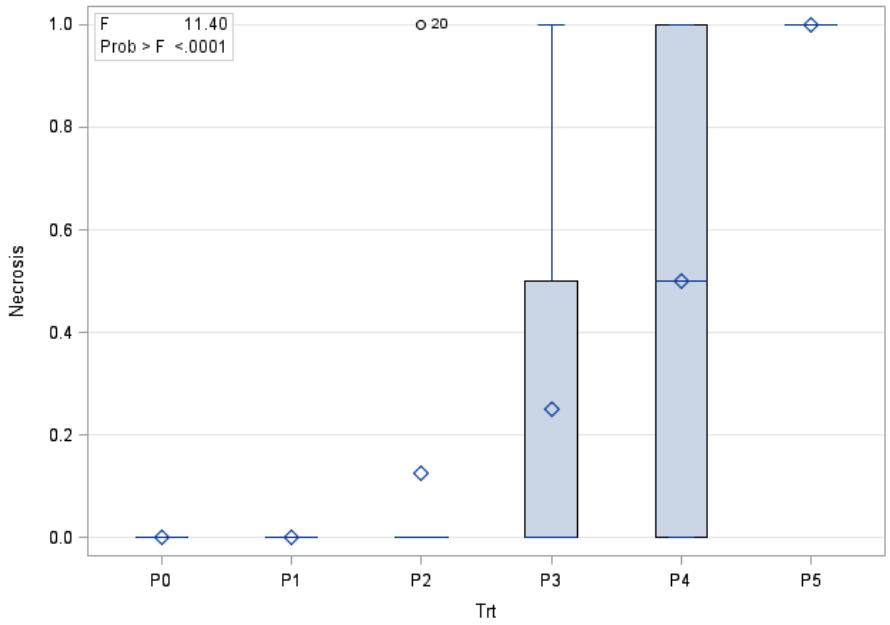


Figure 6.r. Mean necrotic observation distribution per treatment (Trt) after the first growing season. (P0) Control, (P1) 250 mg $kg^{-1}$, (P2) 500 mg $kg^{-1}$, (P3) 750 mg $kg^{-1}$, (P4) 1000 mg $kg^{-1}$, and (P5) 1250 mg $kg^{-1}$.

Significance Comparisons

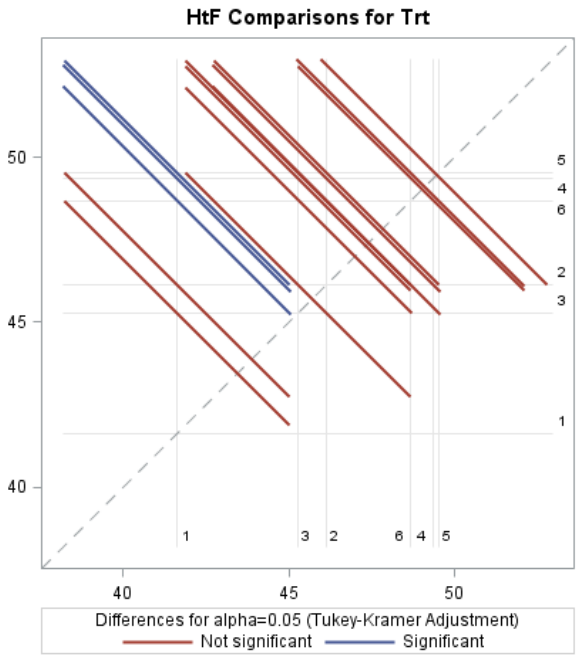


Figure 7.a. Tukey post-hoc test probability for P concentration to affect height growth (HtF) (cm) treatment interaction.

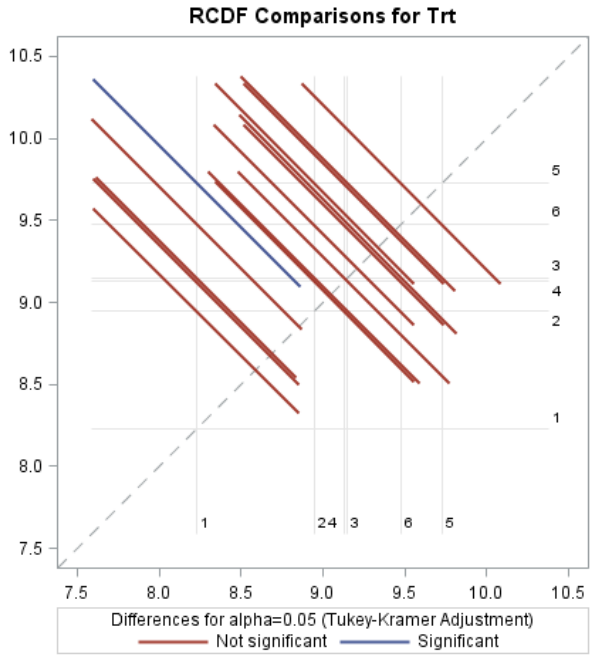


Figure 7.b. Tukey post-hoc test probability for P concentration to affect diameter growth (RCDF) (mm) treatment interaction.

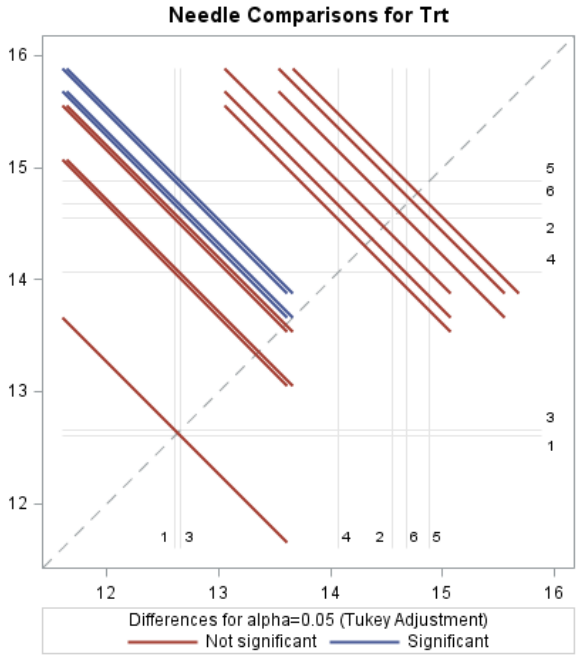


Figure 7.c. Tukey post-hoc test probability for P concentration to affect mean needle length (Needle) (cm) treatment interaction.

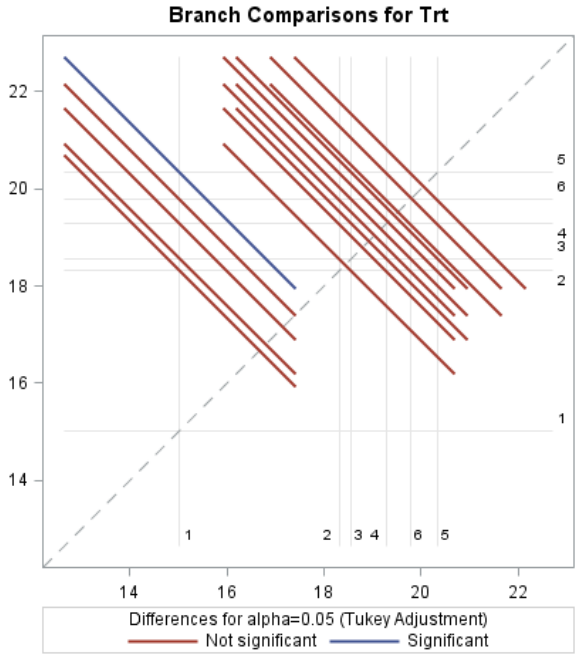


Figure 7.d. Tukey post-hoc test probability for P concentration to affect mean branch length (Branch) (cm) treatment interaction.

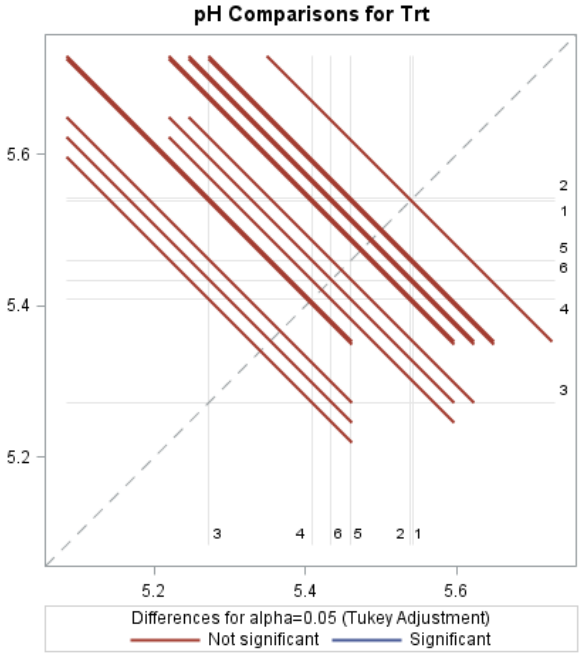


Figure 7.e. Tukey post-hoc test probability for P concentration to affect pH treatment interaction.

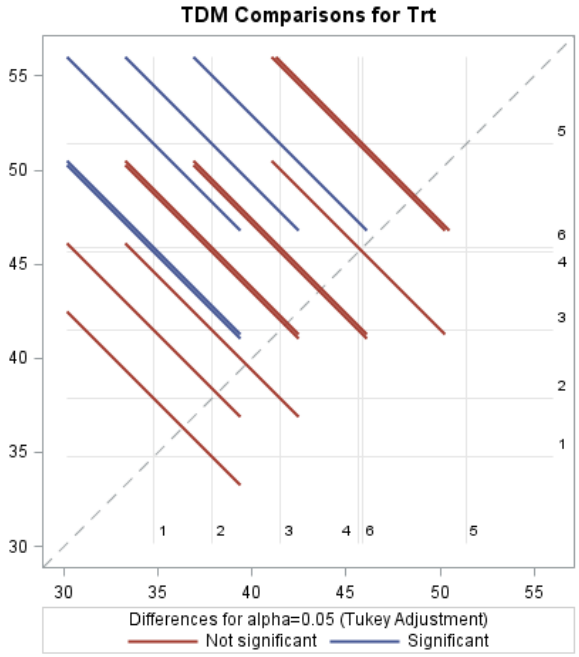


Figure 7.f. Tukey post-hoc test probability for P concentration to affect total dry matter (TDM) (g) treatment interaction.

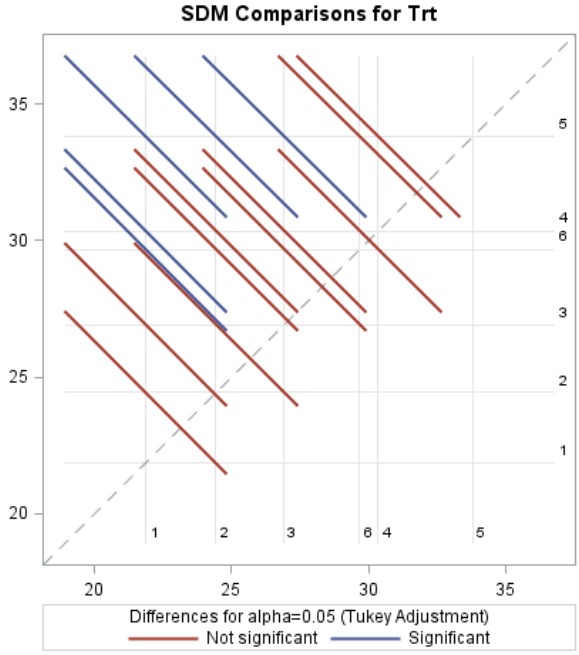


Figure 7.g. Tukey post-hoc test probability for P concentration to affect shoot dry matter (SDM) (g) treatment interaction.

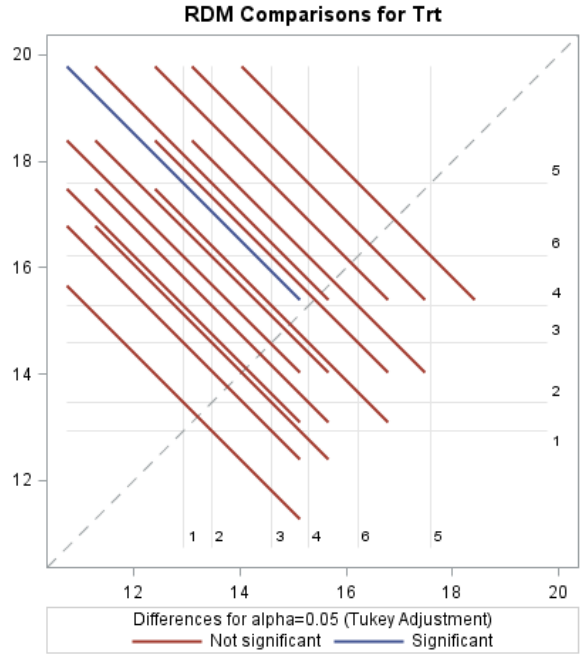


Figure 7.h. Tukey post-hoc test probability for P concentration to affect root dry matter (RDM) (g) treatment interaction.

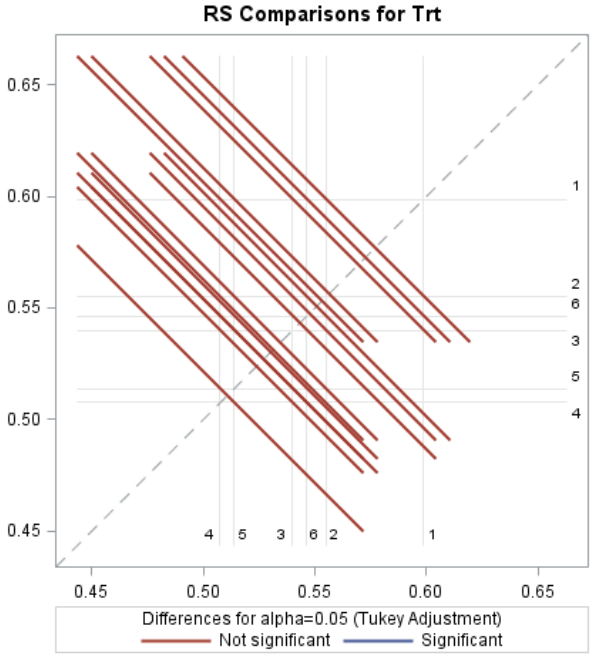


Figure 7.i. Tukey post-hoc test probability for P concentration to affect root-to-shoot ratio (RS) treatment interaction.

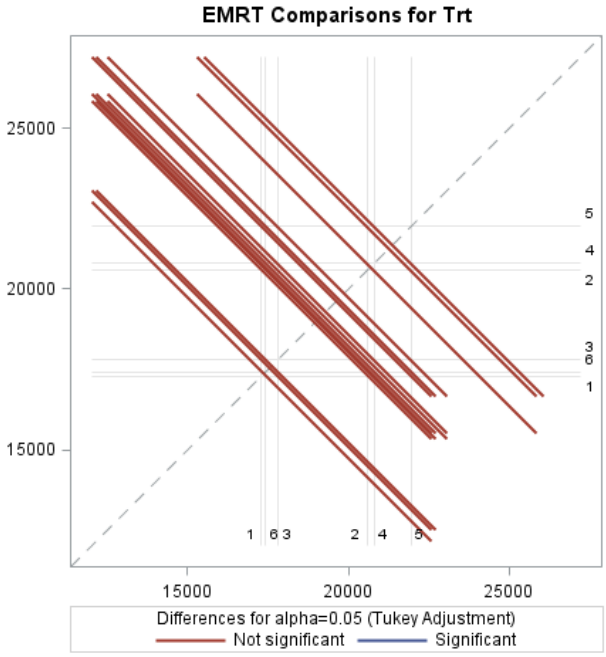


Figure 7.j. Tukey post-hoc test probability for P concentration to affect ectomycorrhizal root tip count (EMRT) treatment interaction.

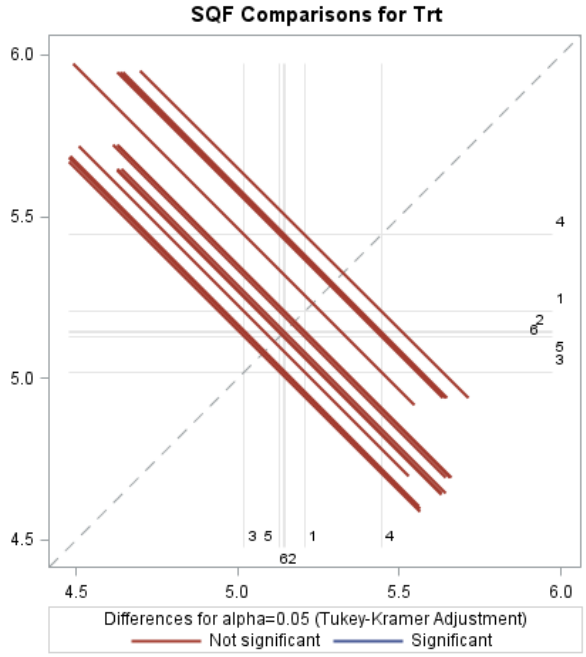


Figure 7.k. Tukey post-hoc test probability for P concentration to affect sturdiness quotient (SQ) treatment interaction.

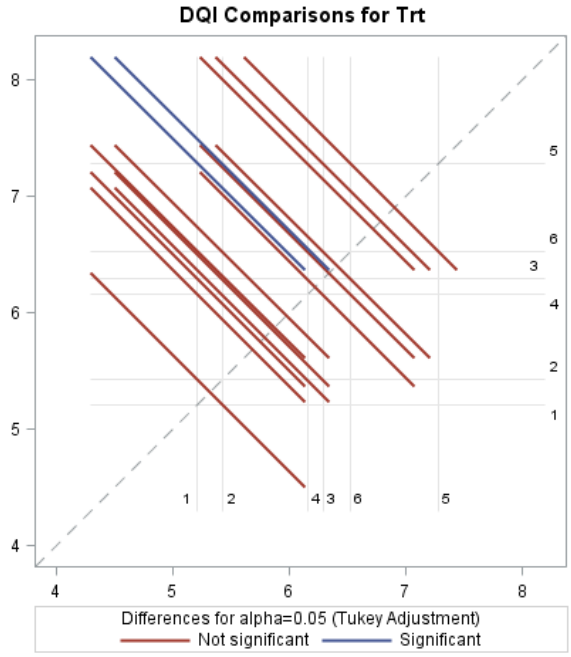


Figure 7.l. Tukey post-hoc test probability for P concentration to affect Dickson quality index (DQI) treatment interaction.

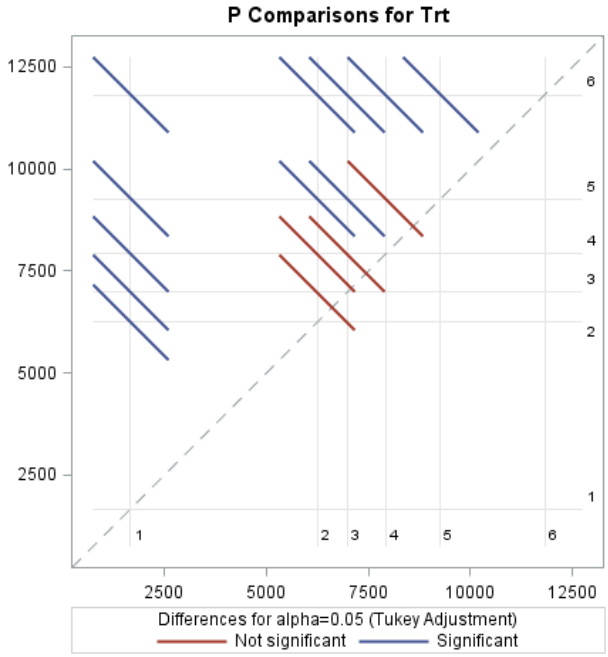


Figure 7.m. Tukey post-hoc test probability for P soil concentration to influence P uptake (mg $kg^{-1}$) treatment interaction.

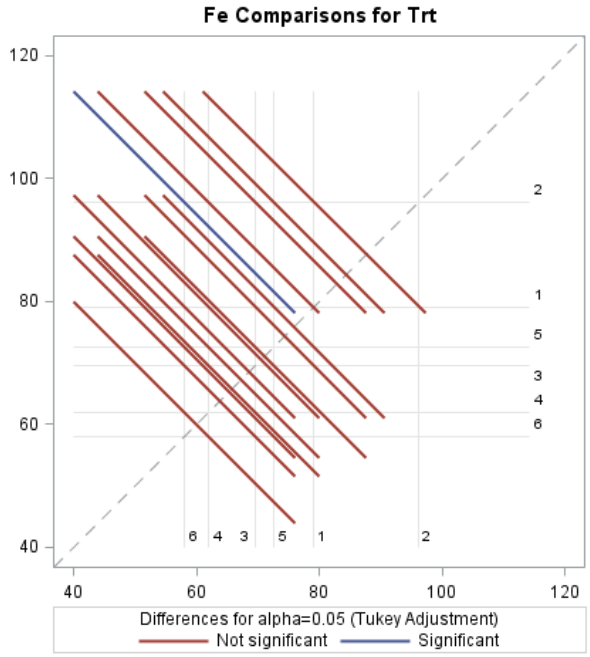


Figure 7.n. Tukey post-hoc test probability for P soil concentration to influence Fe uptake (mg $kg^{-1}$) treatment interaction.

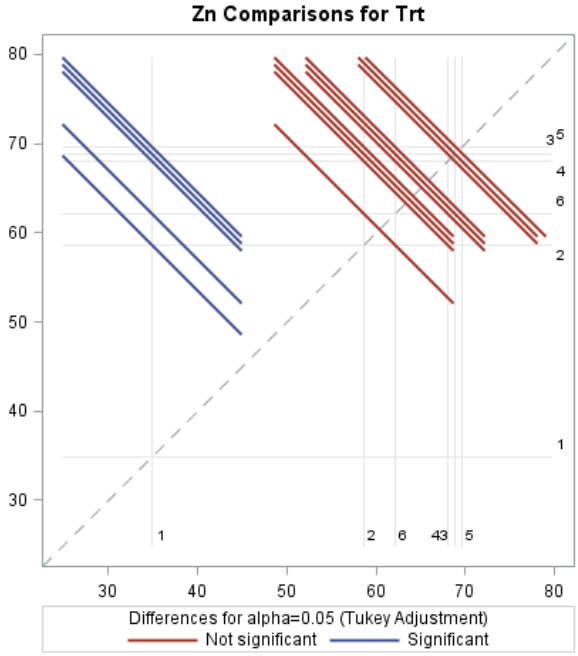


Figure 7.o. Tukey post-hoc test probability for P soil concentration to influence Zn uptake (mg $kg^{-1}$) treatment interaction.

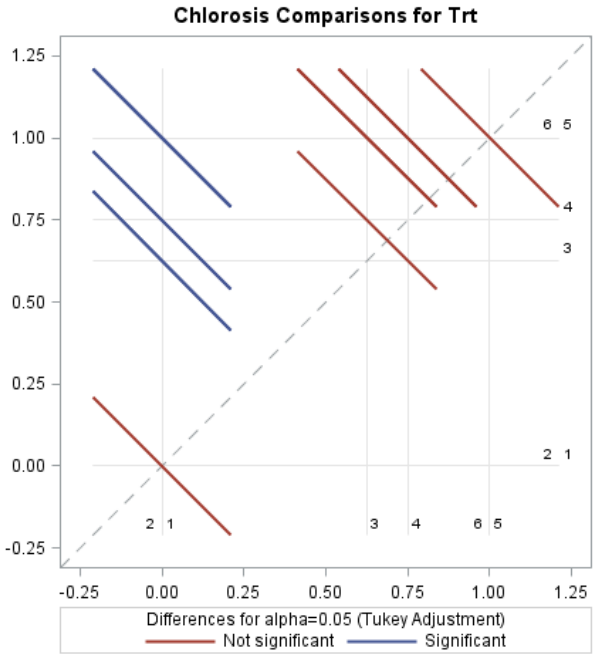


Figure 7.p. Tukey post-hoc test probability for P concentration to affect plant tissue chlorosis treatment interaction.

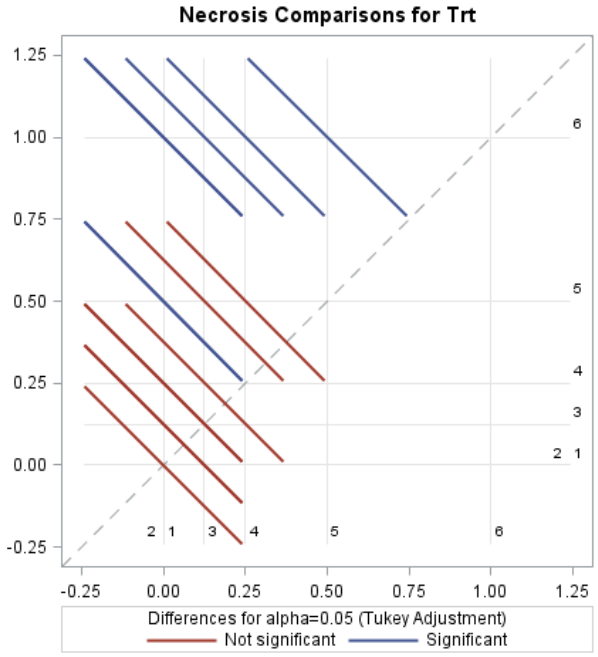


Figure 7.q. Tukey post-hoc test probability for P concentration to affect plant tissue necrosis treatment interaction.

Analysis of Covariance

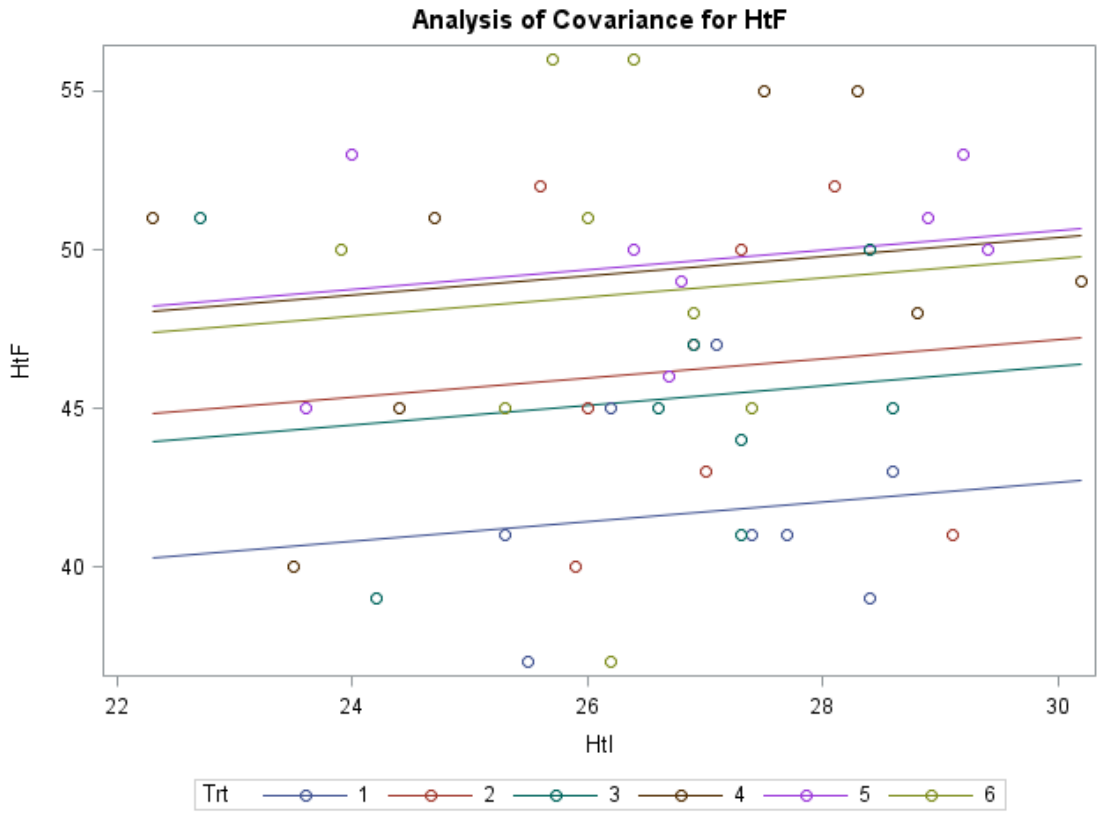


Figure 8.a. Analysis of covariance between height growth after one growing season (HtF), and initial height and treatment (Trt). (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

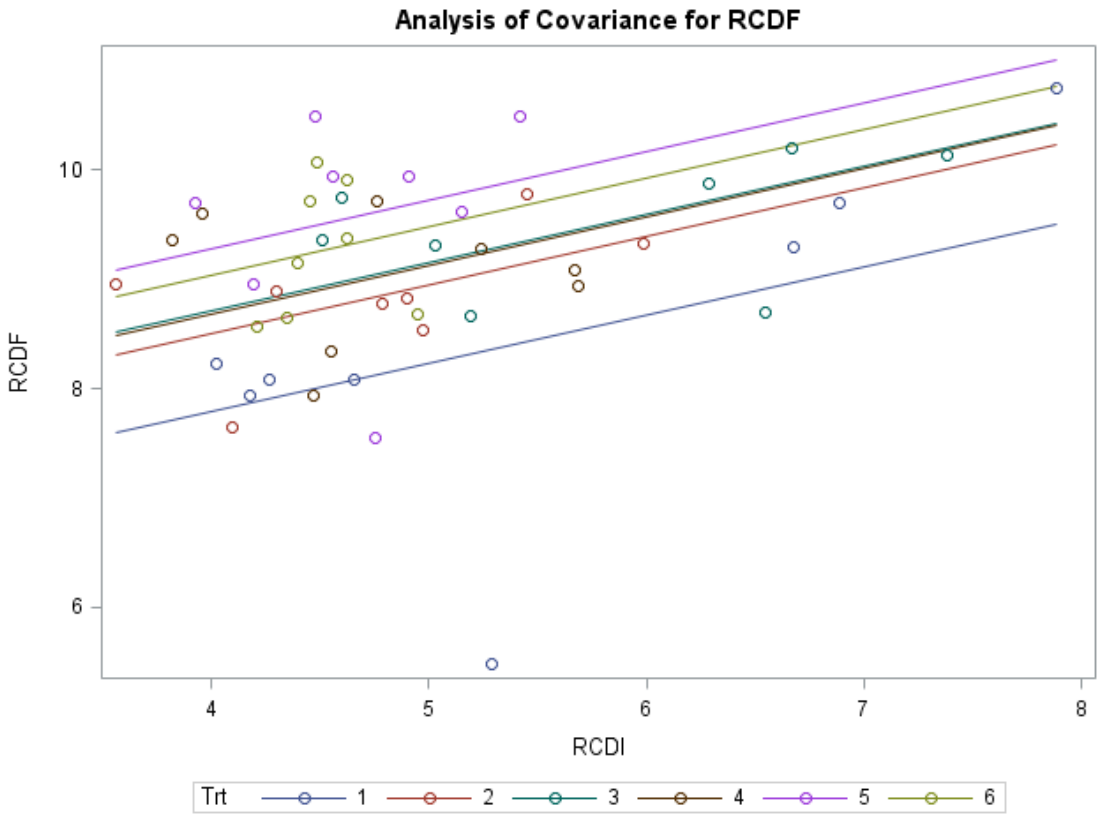


Figure 8.b. Analysis of covariance between root collar diameter growth after one growing season (RCDF), and initial root collar diameter (RCDI) and treatment (Trt). (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

Scatterplots

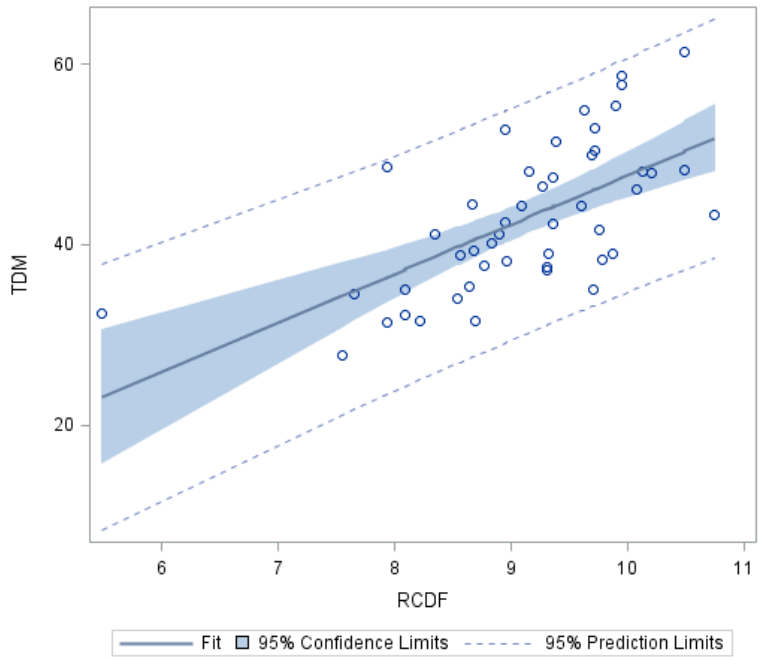


Figure 9. Linear relationship between total dry matter (TDM) (g) and diameter growth (RCDF) (mm) after one growing season.

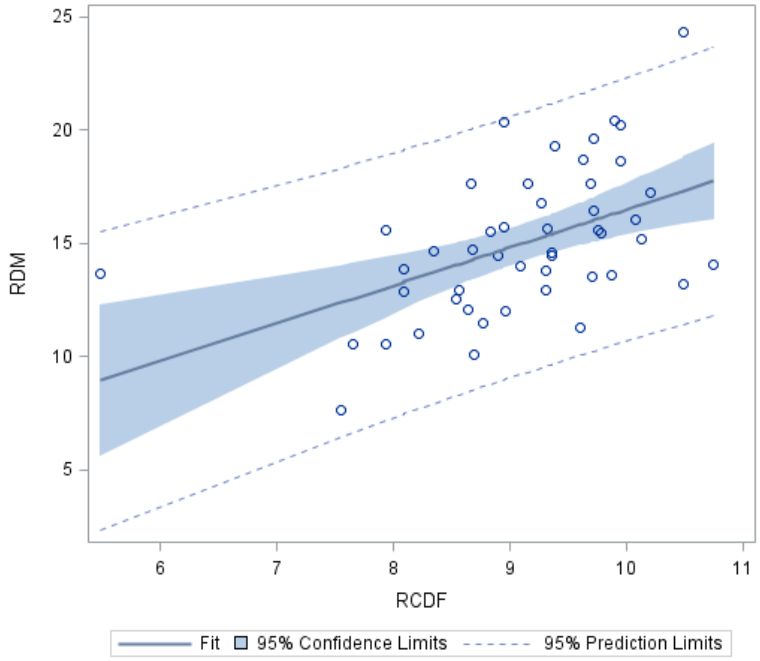


Figure 10. Linear relationship between root dry matter (RDM) (g) and diameter growth (RCDF) (mm) after one growing season.

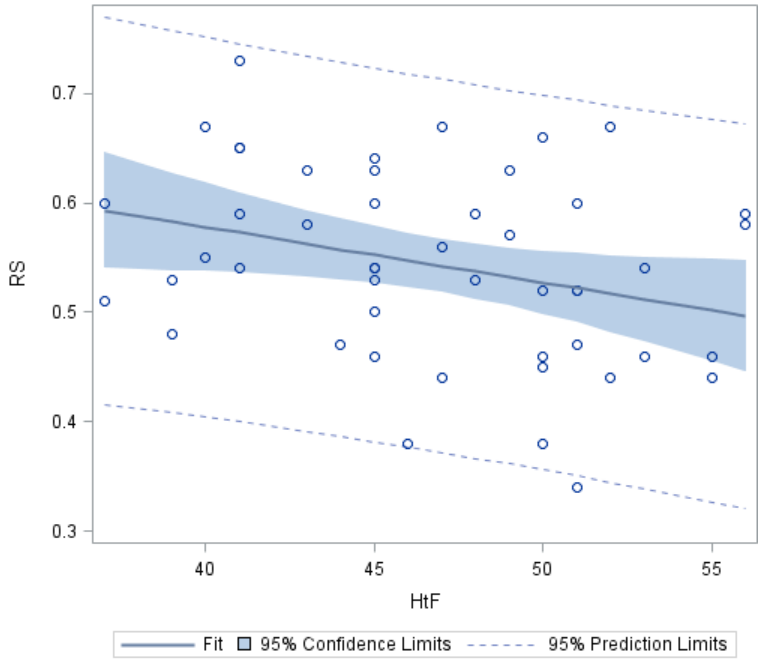


Figure 11. Linear relationship between root-to-shoot ratio (RS) and height growth (HtF) (cm) after one growing season.

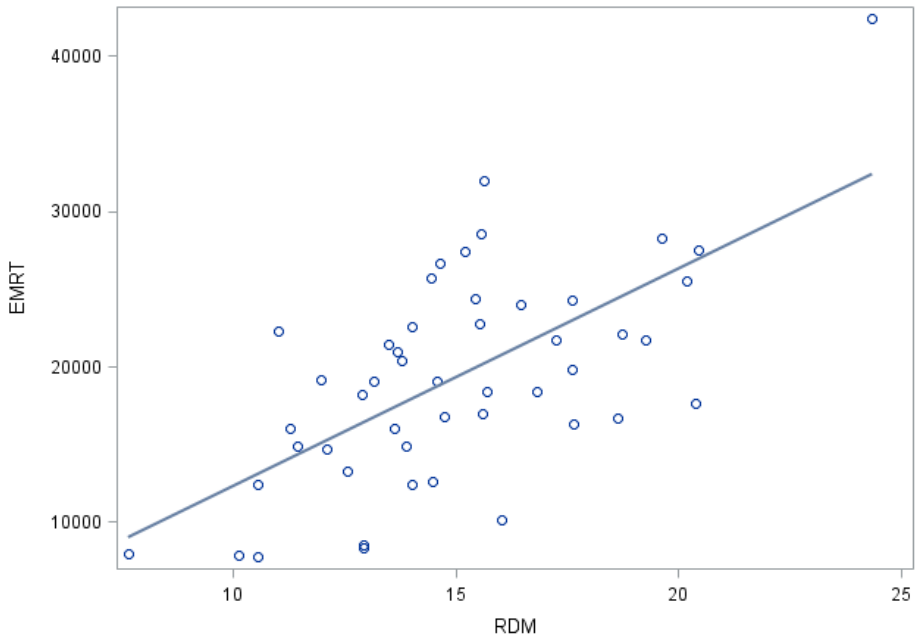


Figure 12. Linear relationship between ectomycorrhizal root tip count (EMRT) and root dry matter (RDM) (g) after one growing season.

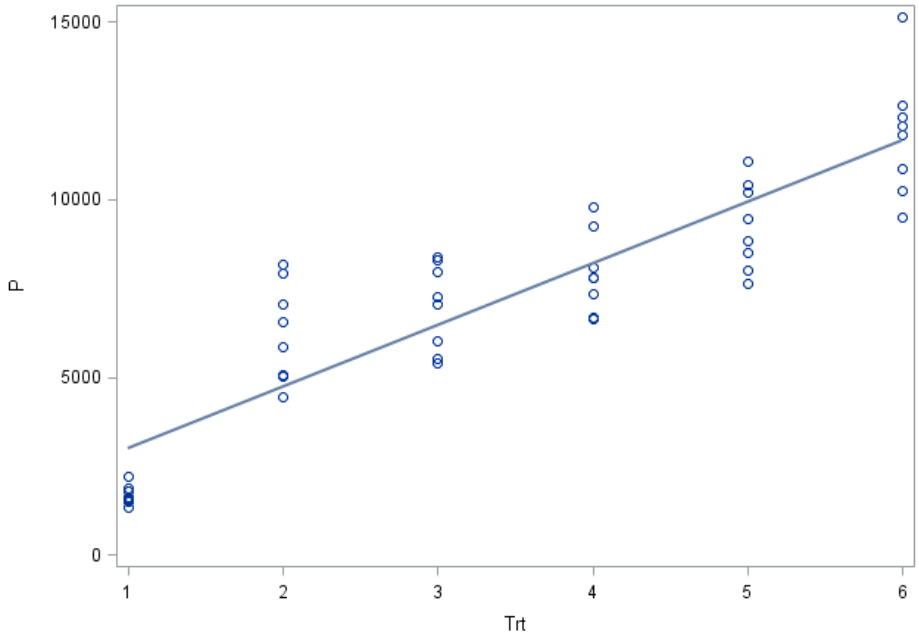


Figure 13. Linear relationship between phosphorus uptake (P) (mg $kg^{-1}$)and treatment level (Trt) after one growing season. (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

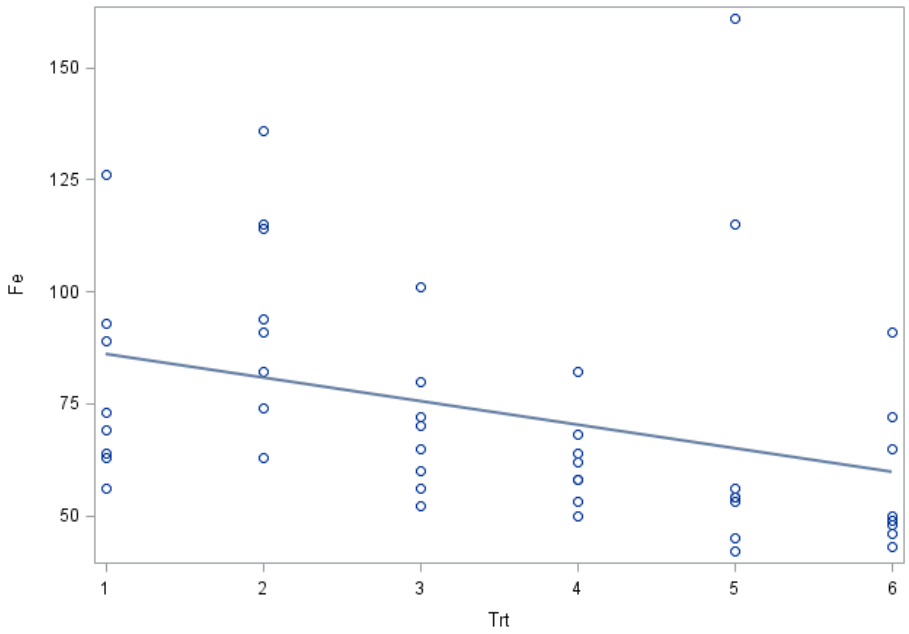


Figure 14. Linear relationship between iron uptake (Fe) (mg $kg^{-1}$) and treatment level (Trt) after one growing season. (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

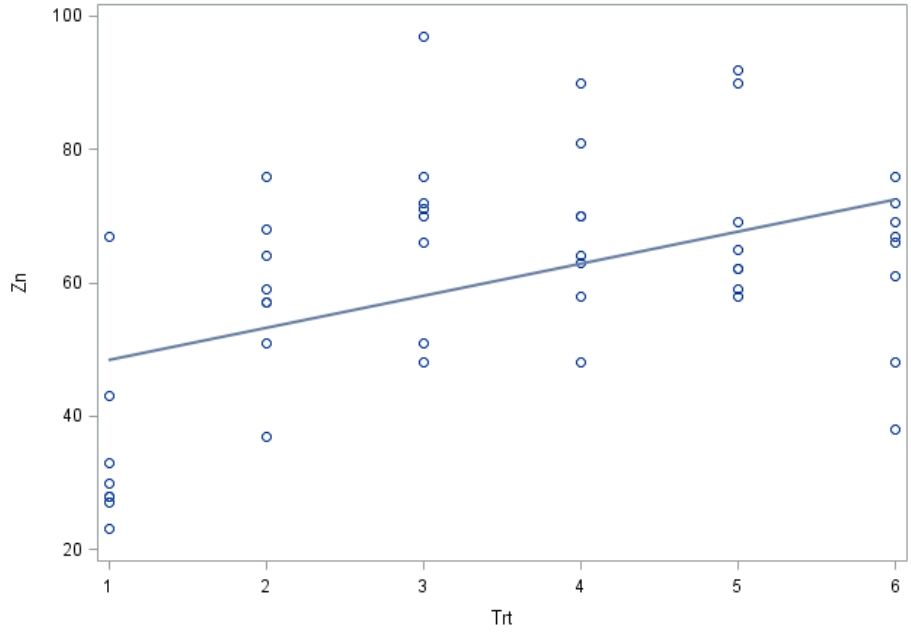


Figure 15. Linear relationship between zinc uptake (Zn) (mg $kg^{-1}$) and treatment level (Trt) after one growing season. (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

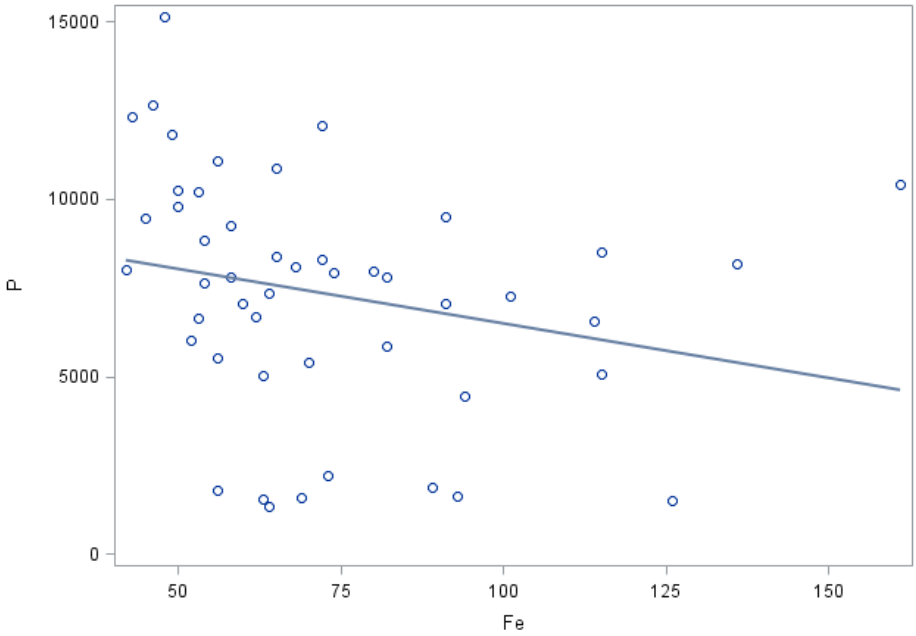


Figure 16. Linear relationship between phosphorus uptake (P) and iron uptake (Fe) after one growing season.

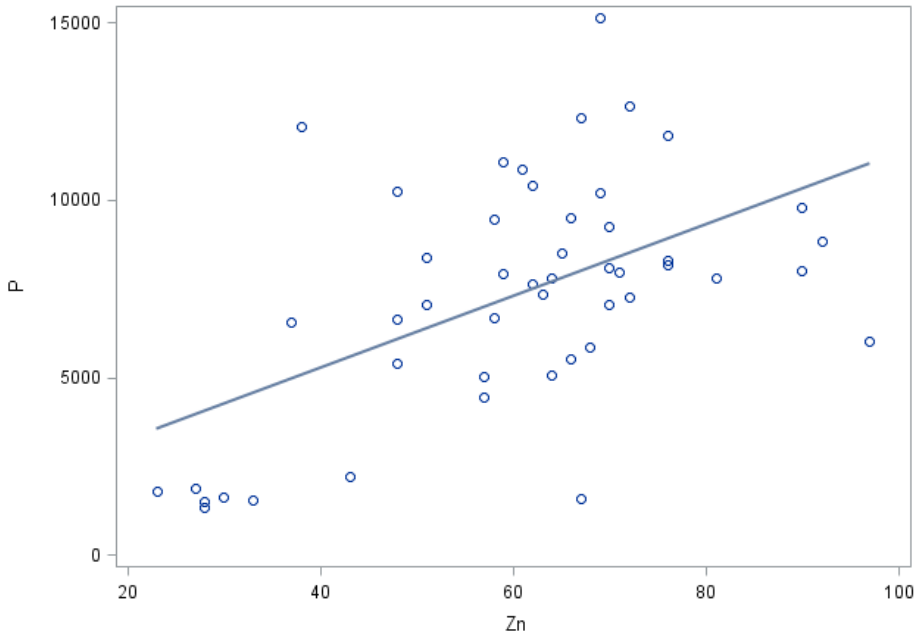


Figure 17. Linear relationship between phosphorus uptake (P) and zinc uptake (Zn) after one growing season.

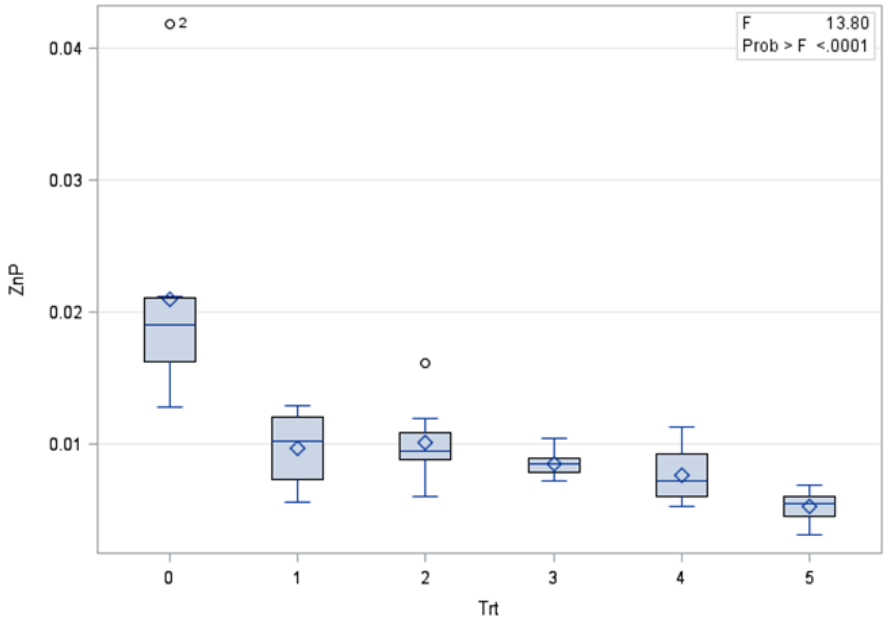


Figure 18. Zinc-to-phosphorus ratio distribution by treatment after one growing season. (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

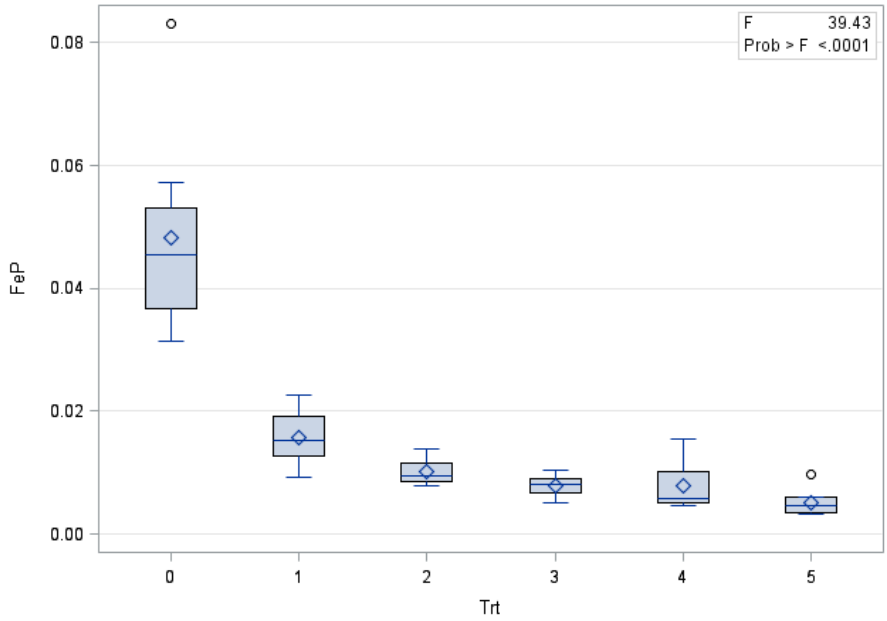


Figure 19. Iron-to-phosphorus ratio distribution by treatment after one growing season. (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$.

Fit Diagnostics

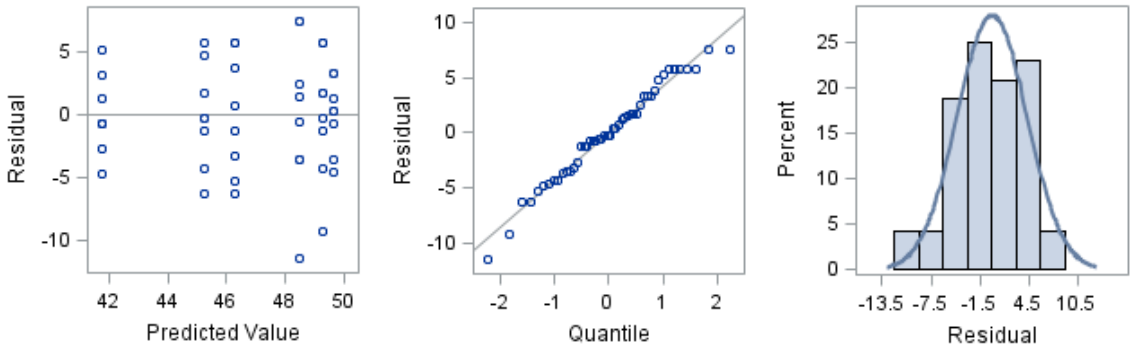


Figure 20.a-c. Fit diagnostic criteria for height growth including: equal variance (left), independence (middle), and normal distributions (right).

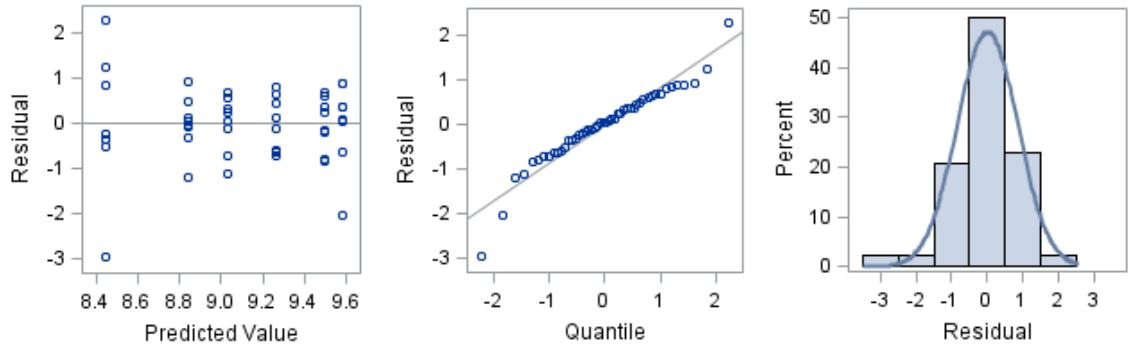


Figure 21.a-c. Fit diagnostic criteria for diameter growth including: equal variance (left), independence (middle), and normal distributions (right).

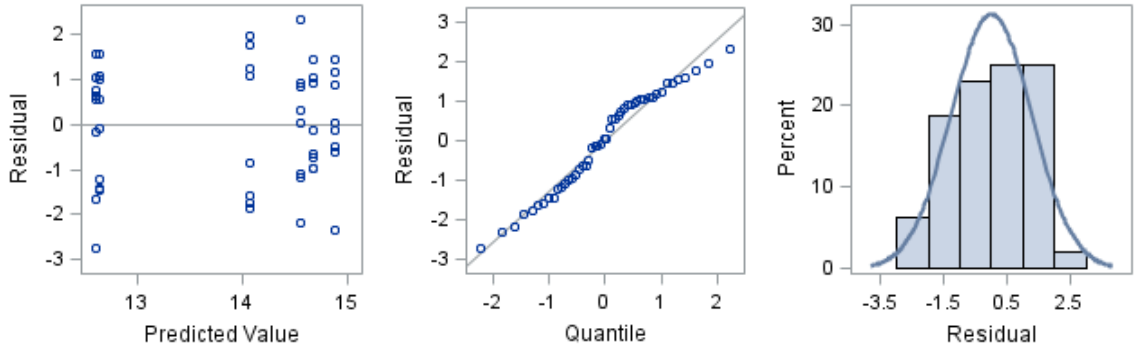


Figure 22.a-c. Fit diagnostic criteria for needle length including: equal variance (left), independence (middle), and normal distributions (right).

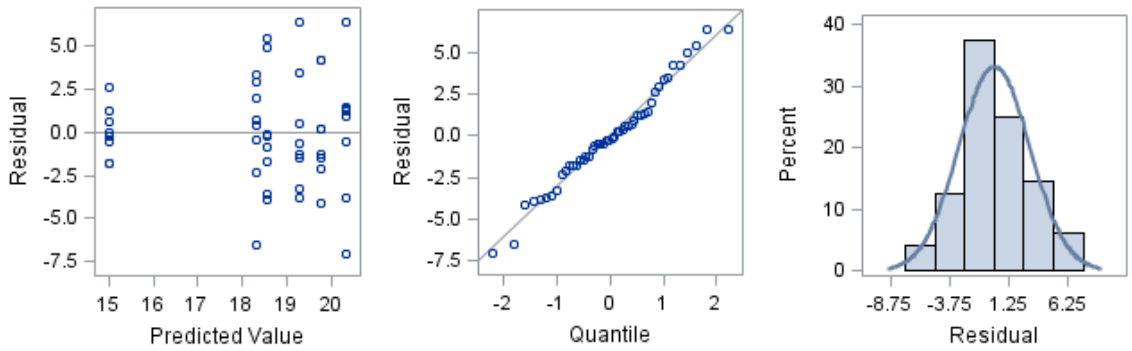


Figure 23.a-c. Fit diagnostic criteria for branch length including: equal variance (left), independence (middle), and normal distributions (right).

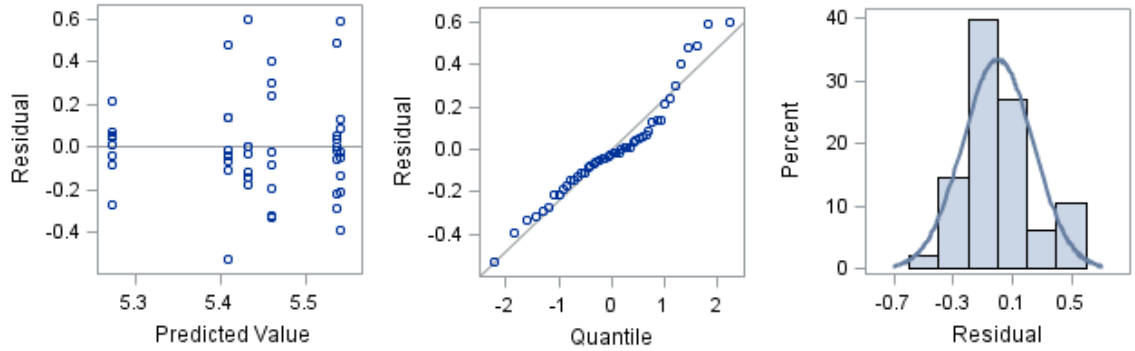


Figure 24.a-c. Fit diagnostic criteria for pH including: equal variance (left), independence (middle), and normal distributions (right).

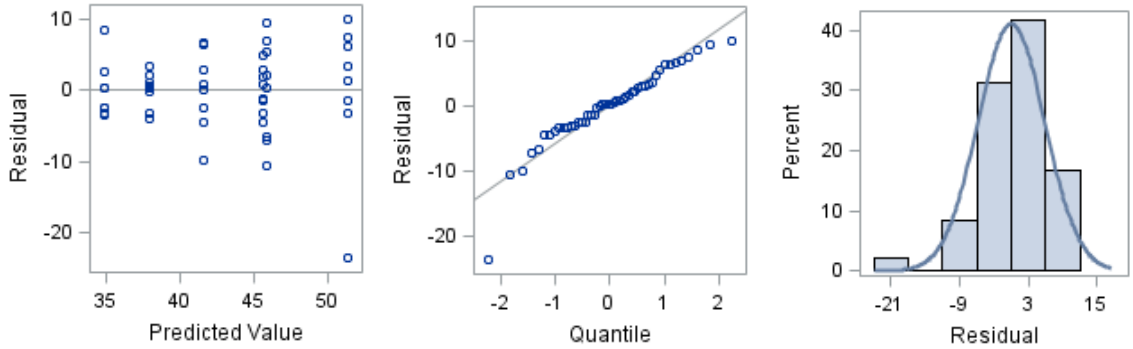


Figure 25.a-c. Fit diagnostic criteria for total dry matter including: equal variance (left), independence (middle), and normal distributions (right).

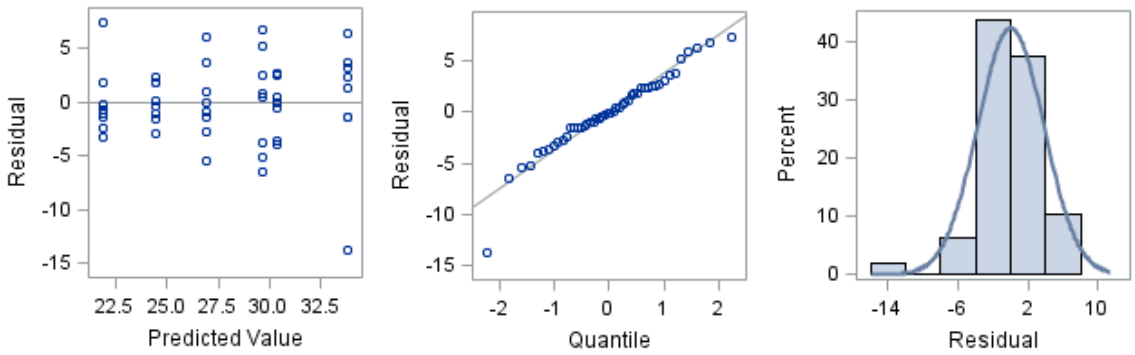


Figure 26.a-c. Fit diagnostic criteria for shoot dry matter including: equal variance (left), independence (middle), and normal distributions (right).

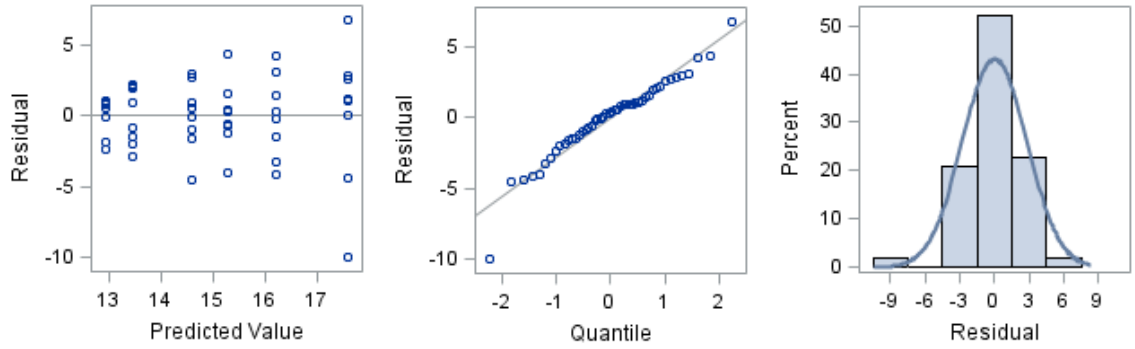


Figure 27.a-c. Fit diagnostic criteria for root dry matter including: equal variance (left), independence (middle), and normal distributions (right).

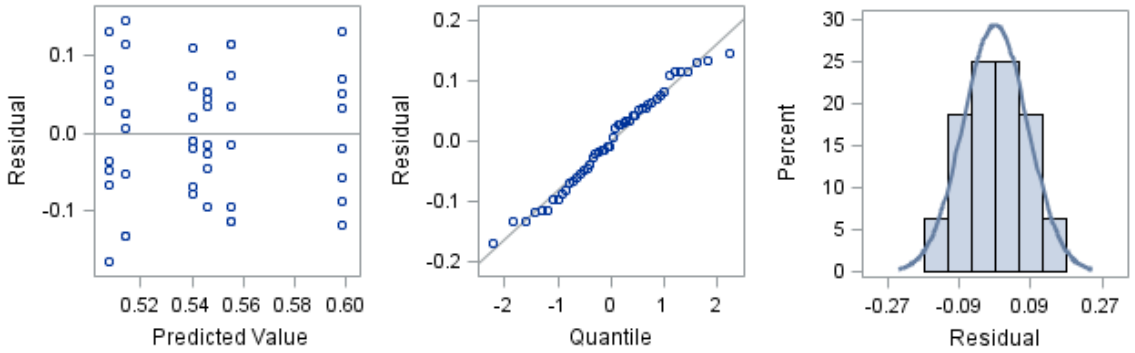


Figure 28.a-c. Fit diagnostic criteria for root-to-shoot ratio including: equal variance (left), independence (middle), and normal distributions (right).

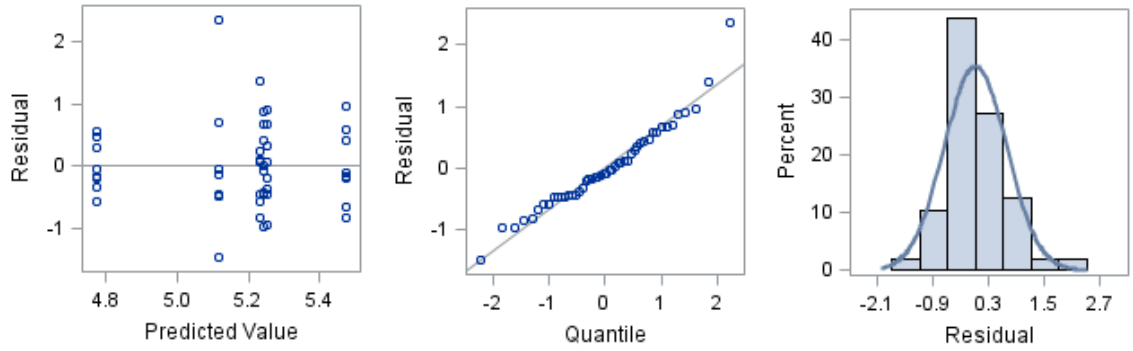


Figure 29.a-c. Fit diagnostic criteria for sturdiness quotient including: equal variance (left), independence (middle), and normal distributions (right).

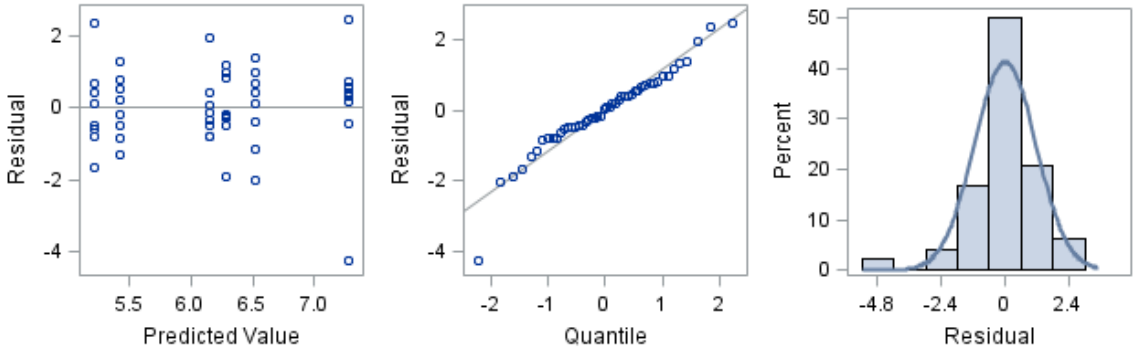


Figure 30.a-c. Fit diagnostic criteria for Dickson quality index including: equal variance (left), independence (middle), and normal distributions (right).

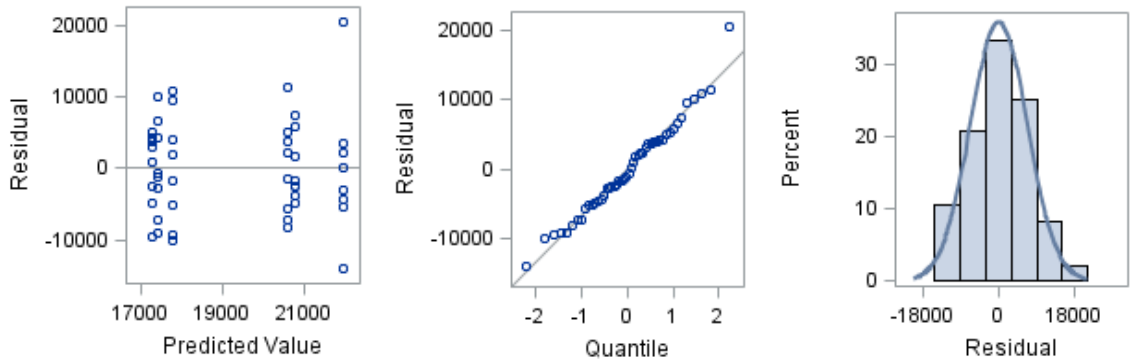


Figure 31.a-c. Fit diagnostic criteria for ectomycorrhizal root tip count including: equal variance (left), independence (middle), and normal distributions (right).

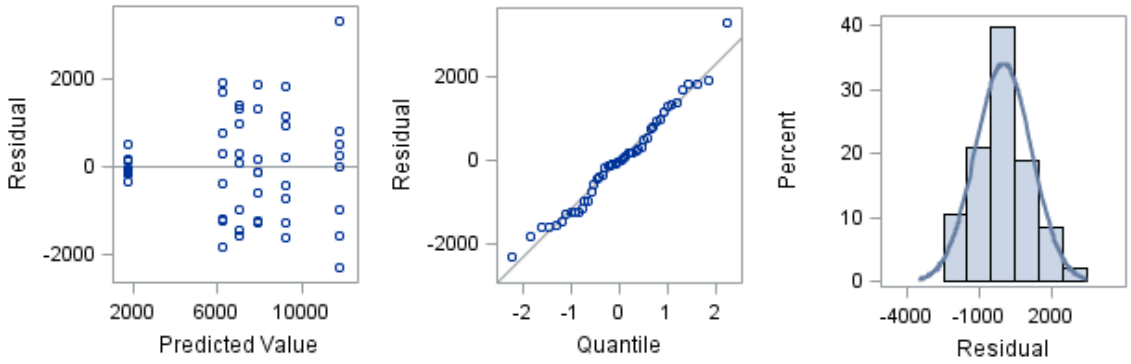


Figure 32.a-c. Fit diagnostic criteria for phosphorus uptake including: equal variance (left), independence (middle), and normal distributions (right).

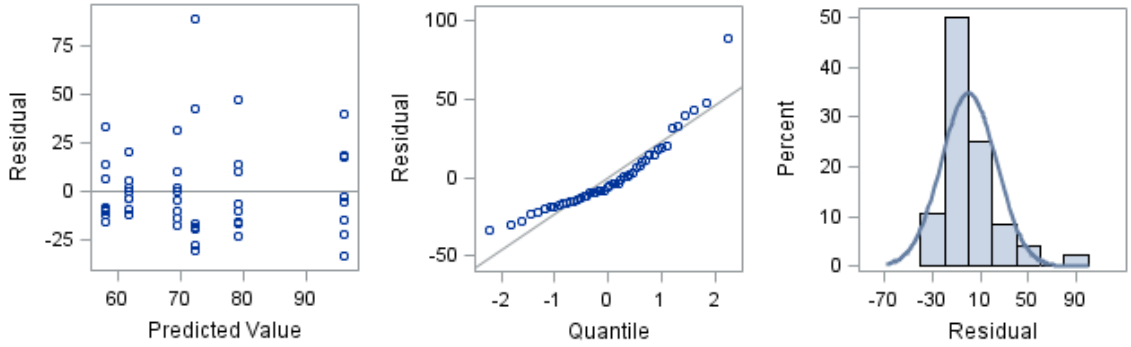


Figure 33.a-c. Fit diagnostic criteria for iron uptake including: equal variance (left), independence (middle), and normal distributions (right).

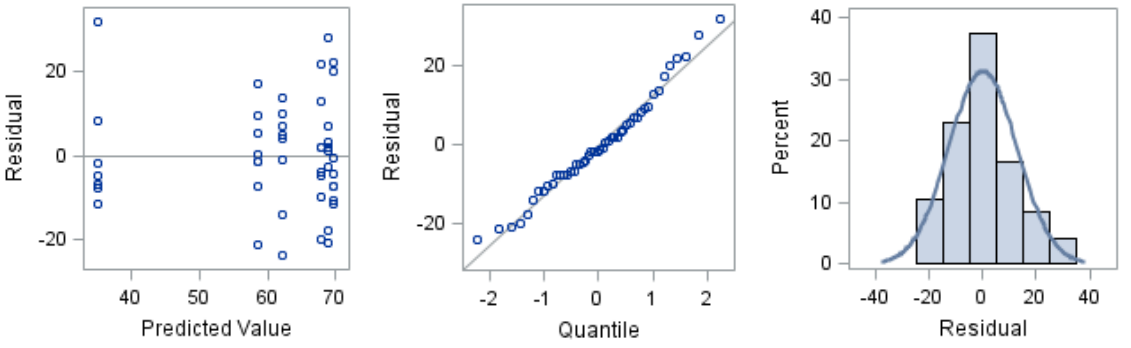


Figure 34.a-c. Fit diagnostic criteria for zinc uptake including: equal variance (left), independence (middle), and normal distributions (right).

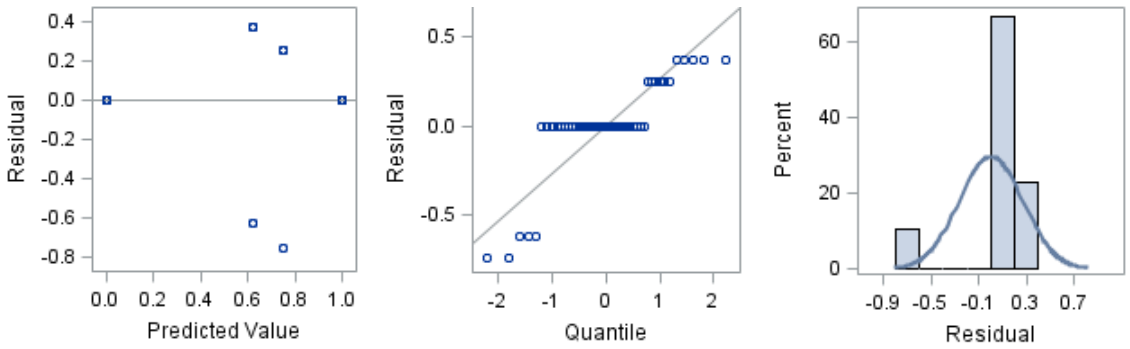


Figure 35.a-c. Fit diagnostic criteria for chlorosis including: equal variance (left), independence (middle), and normal distributions (right).

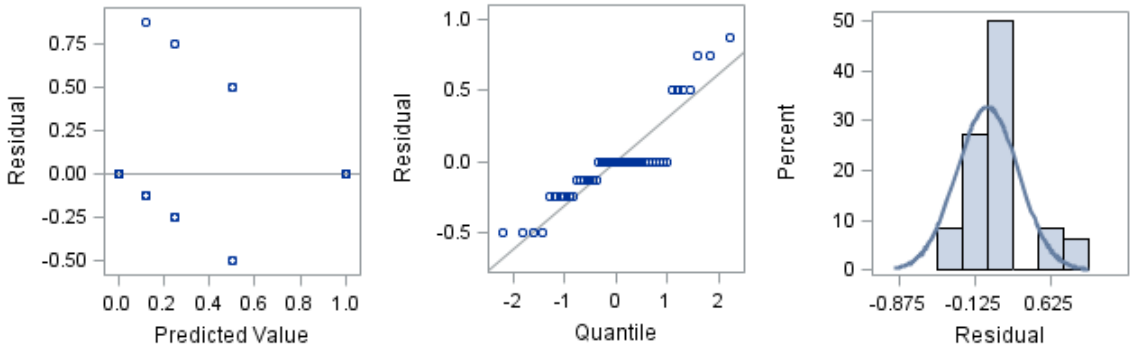


Figure 36.a-c. Fit diagnostic criteria for necrosis including: equal variance (left), independence (middle), and normal distributions (right).

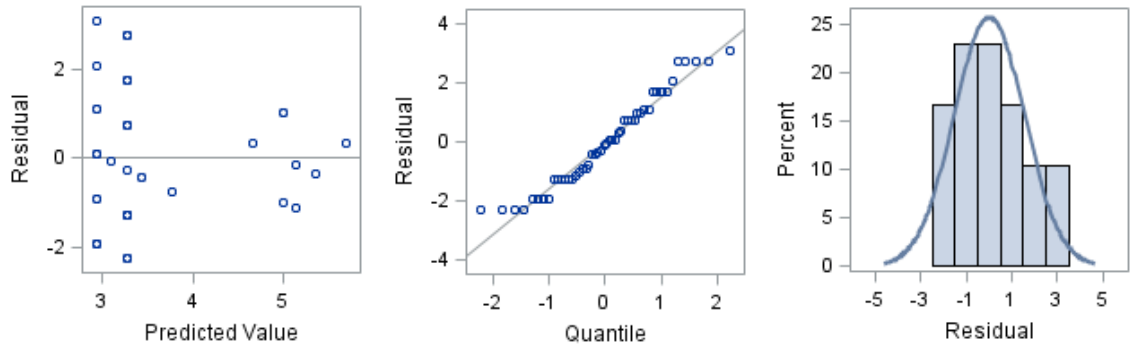


Figure 37.a-c. Fit diagnostic criteria for living Munsell color including: equal variance (left), independence (middle), and normal distributions (right).

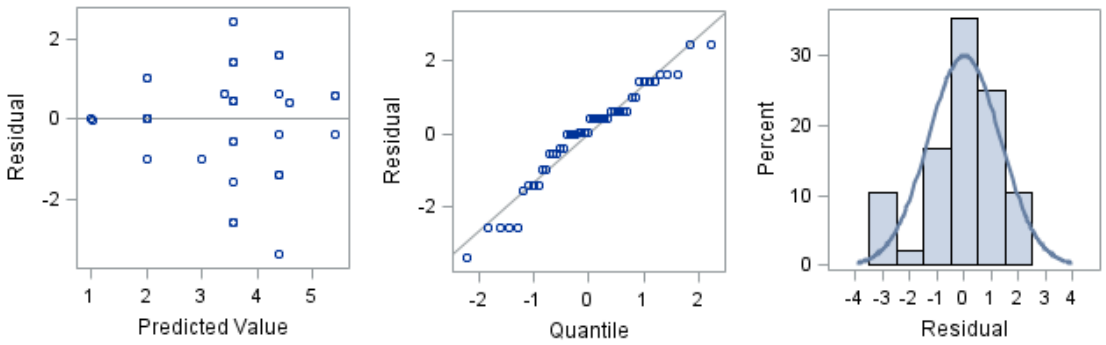


Figure 38.a-c. Fit diagnostic criteria for dried and ground Munsell color including: equal variance (left), independence (middle), and normal distributions (right).

3-Dimensional Color Plot

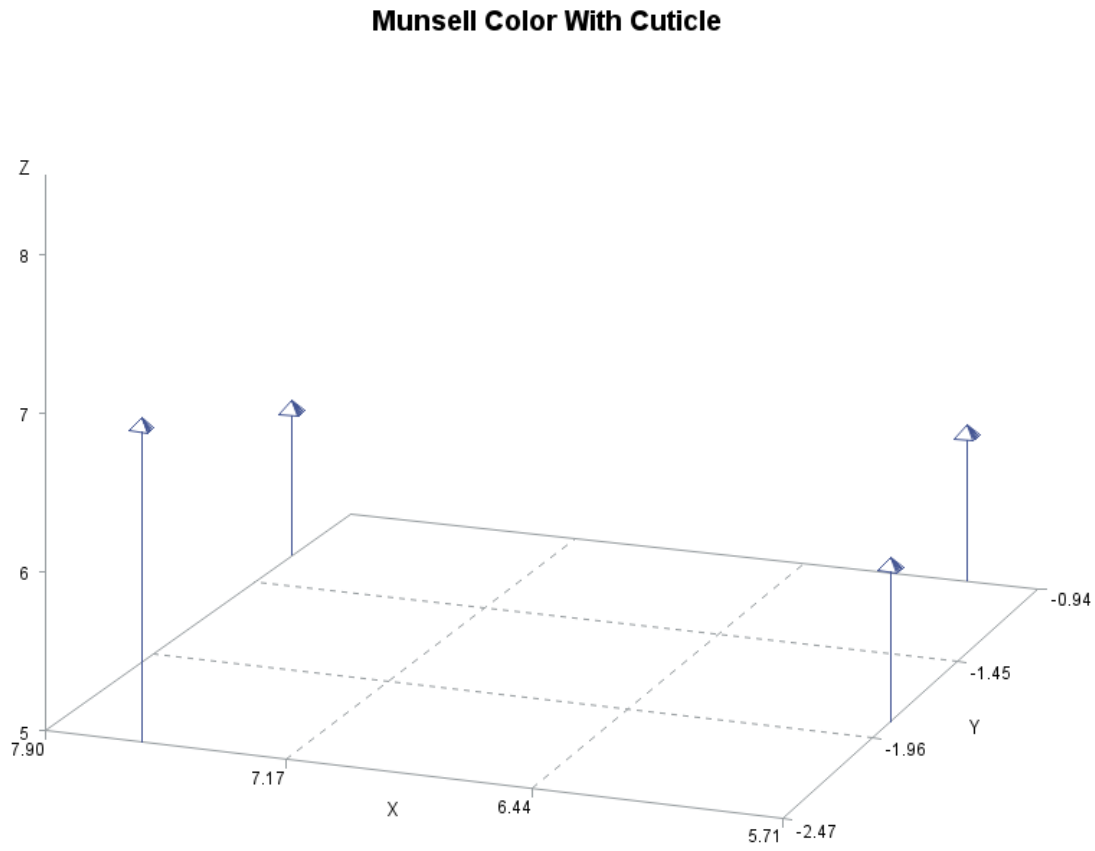


Figure 39. 3-dimensional scatterplot that shows distribution of living Munsell color data.

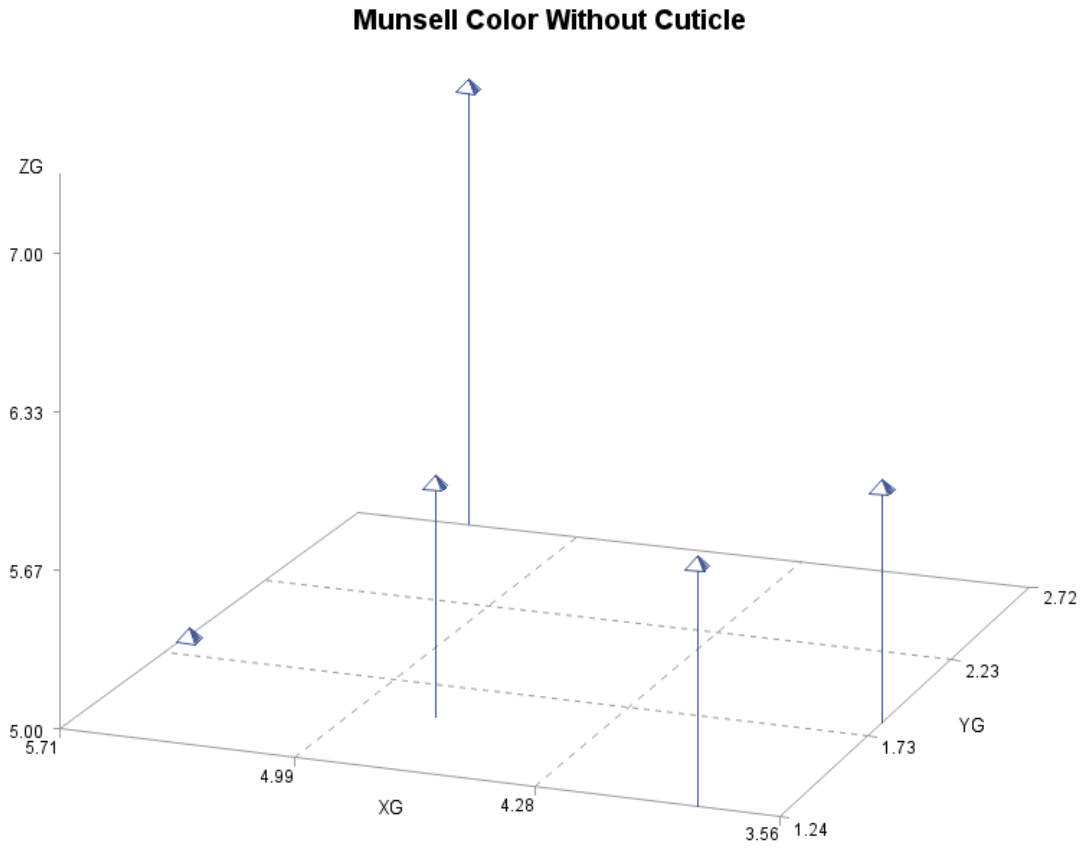


Figure 40. 3-dimensional scatterplot that shows distribution of dried and ground Munsell color data.

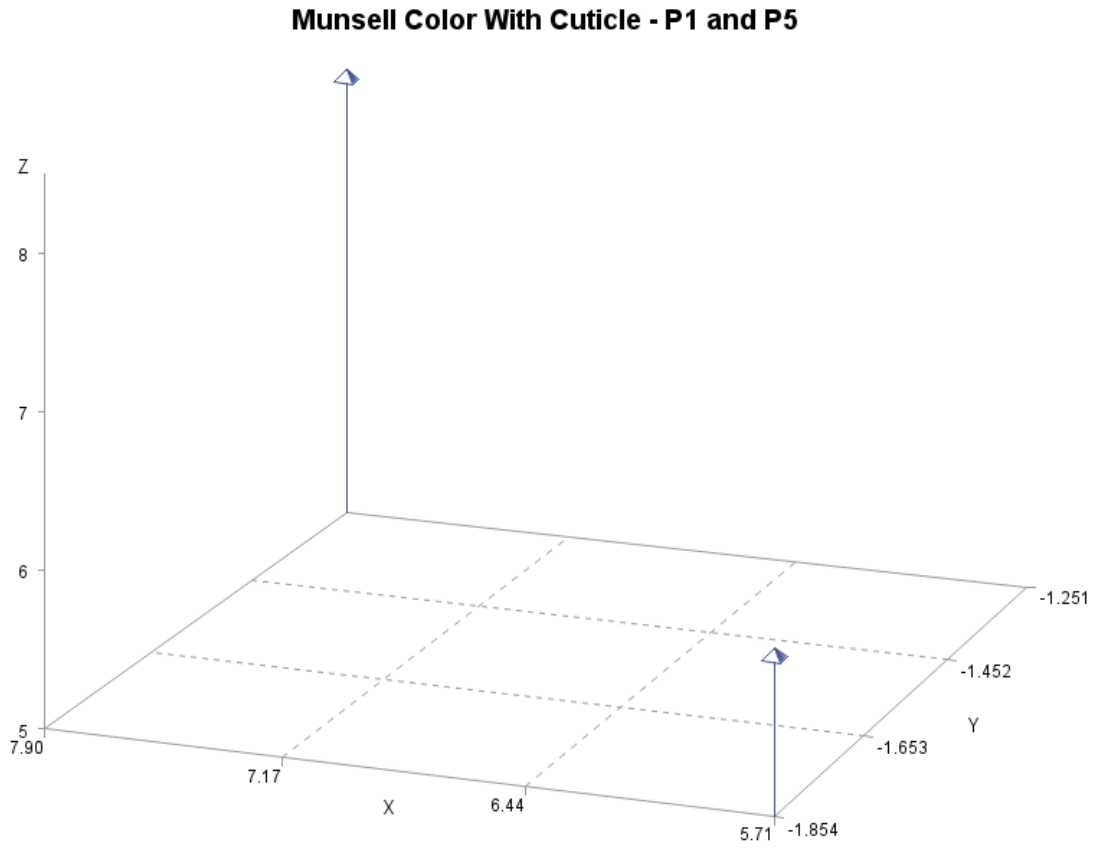


Figure 41. 3-dimensional scatterplot that shows distribution of living Munsell color data from treatments P1 and P5.

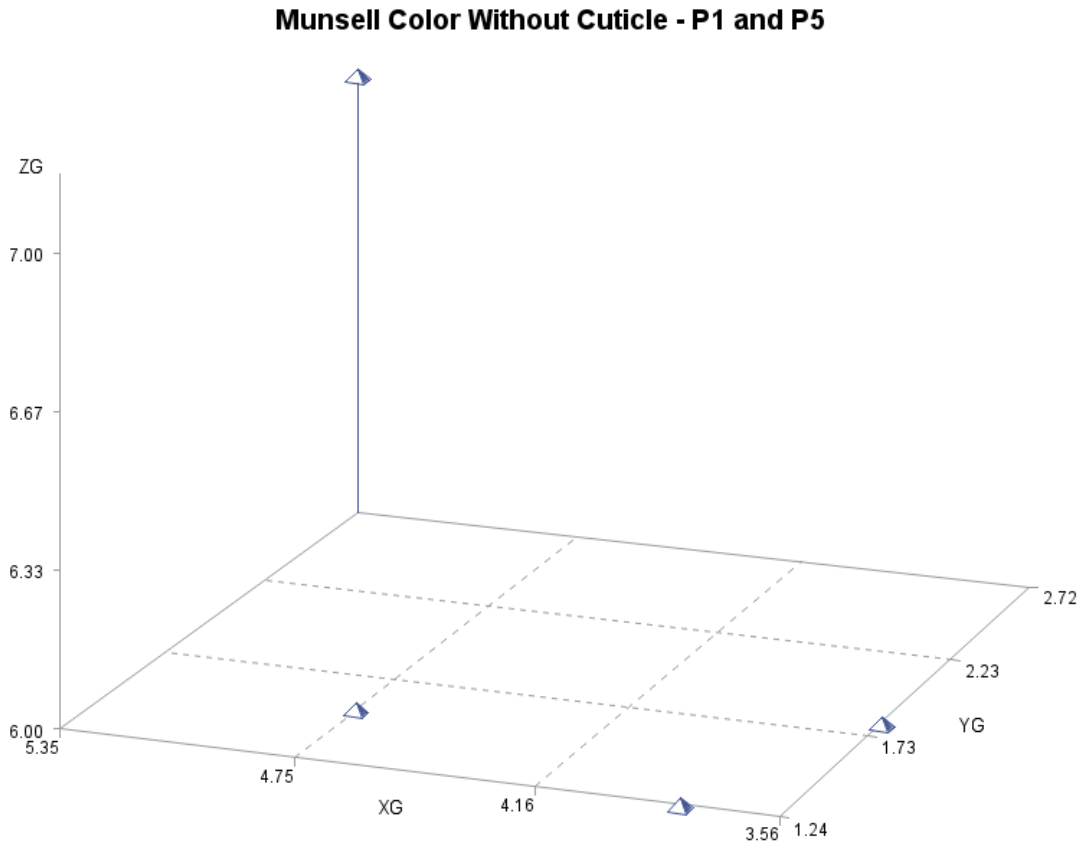


Figure 42. 3-dimensional scatterplot that shows distribution of dried and ground Munsell color data from treatments P1 and P5.

**APPENDIX B –** Photographs

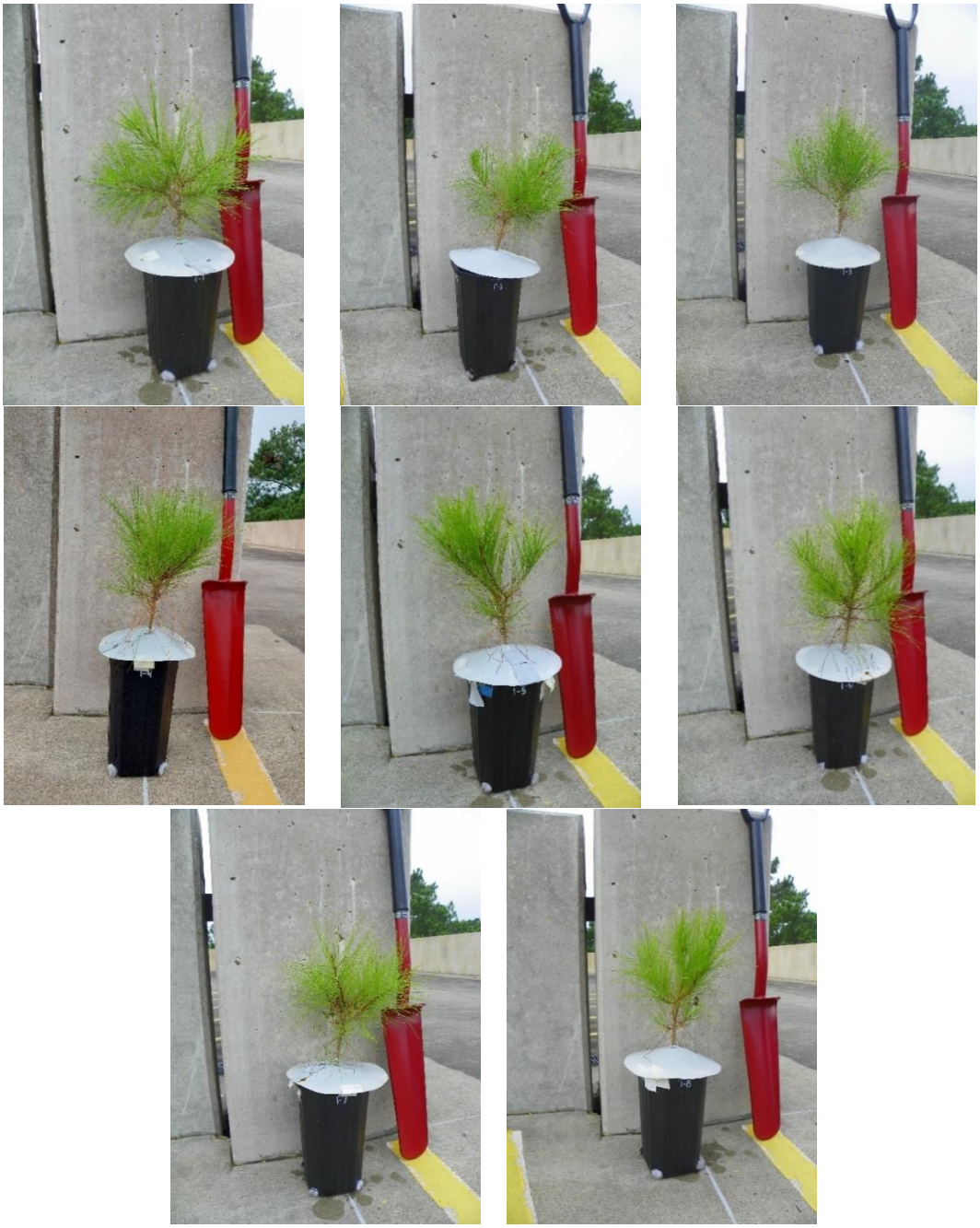

Figure 43. a-h. Photographs of control after one growing season, replications one through eight.

Figure 44. a-h. Photographs of 250 mg $kg^{-1}$ treatment after one growing season, replications one through eight.

Figure 45. a-h. Photographs of 500 mg $kg^{-1}$ treatment after one growing season, replications one through eight.

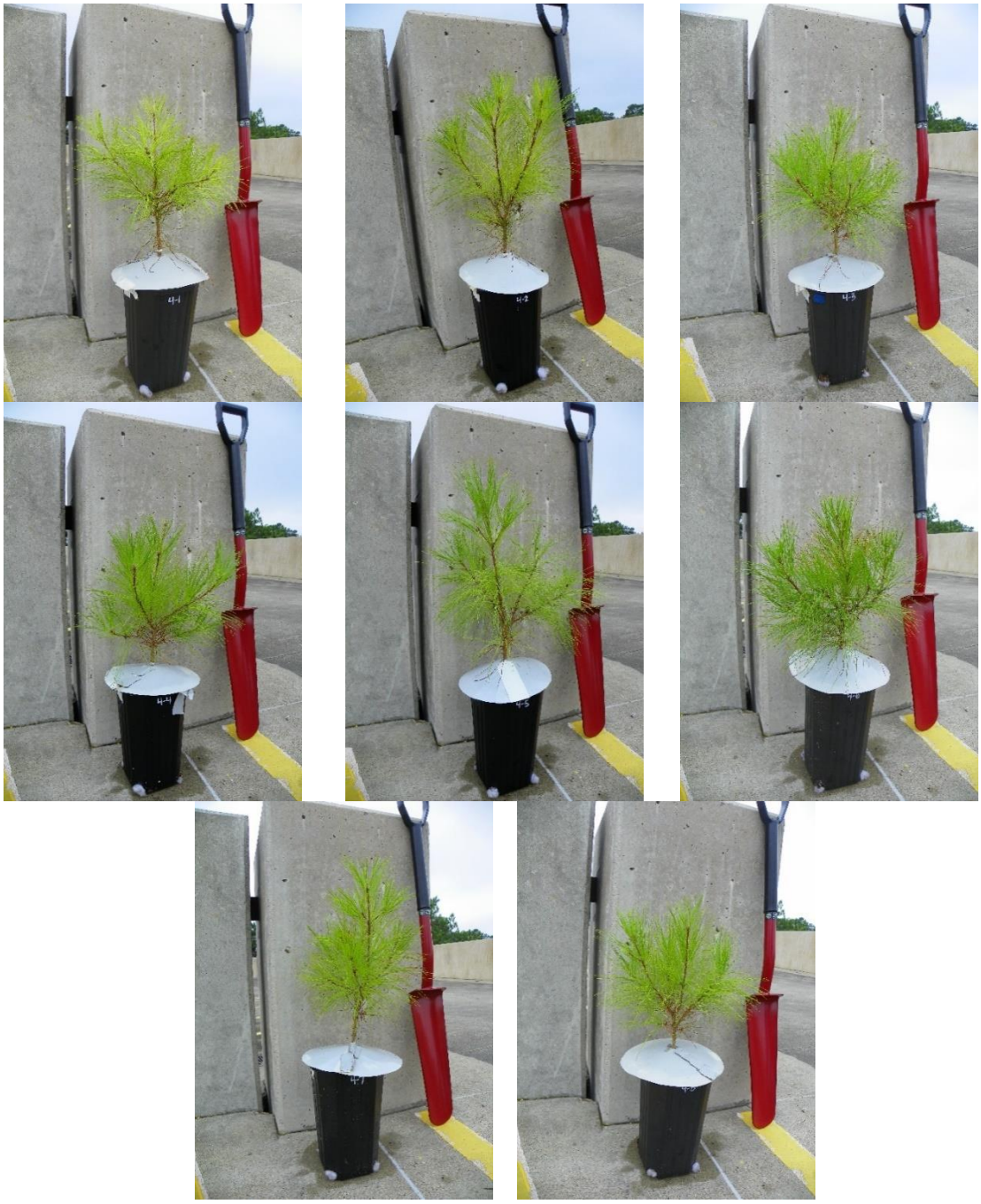

Figure 46. a-h. Photographs of 750 mg $kg^{-1}$ treatment after one growing season, replications one through eight.

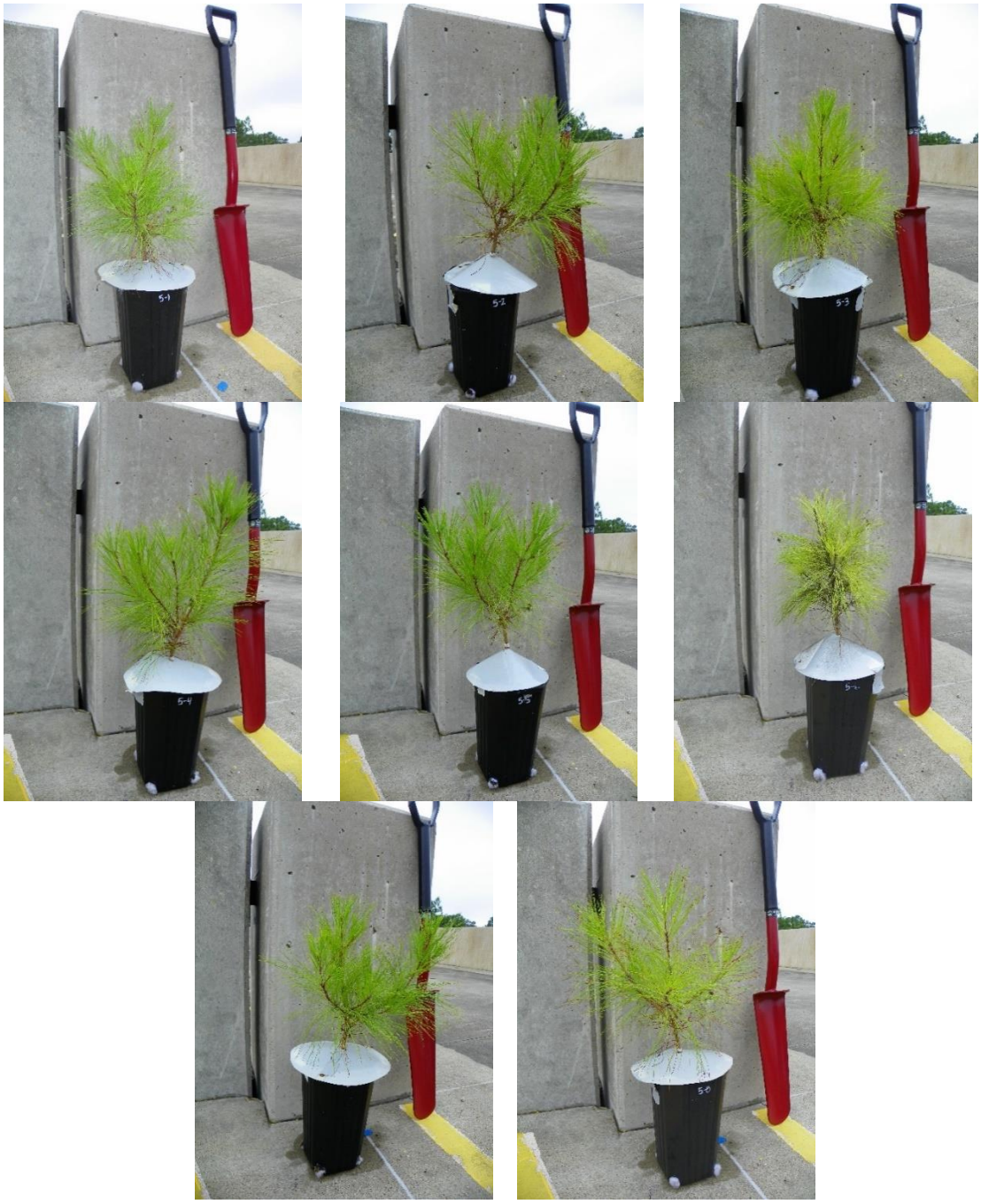

Figure 47. a-h. Photographs of 1000 mg $kg^{-1}$ treatment after one growing season, replications one through eight.

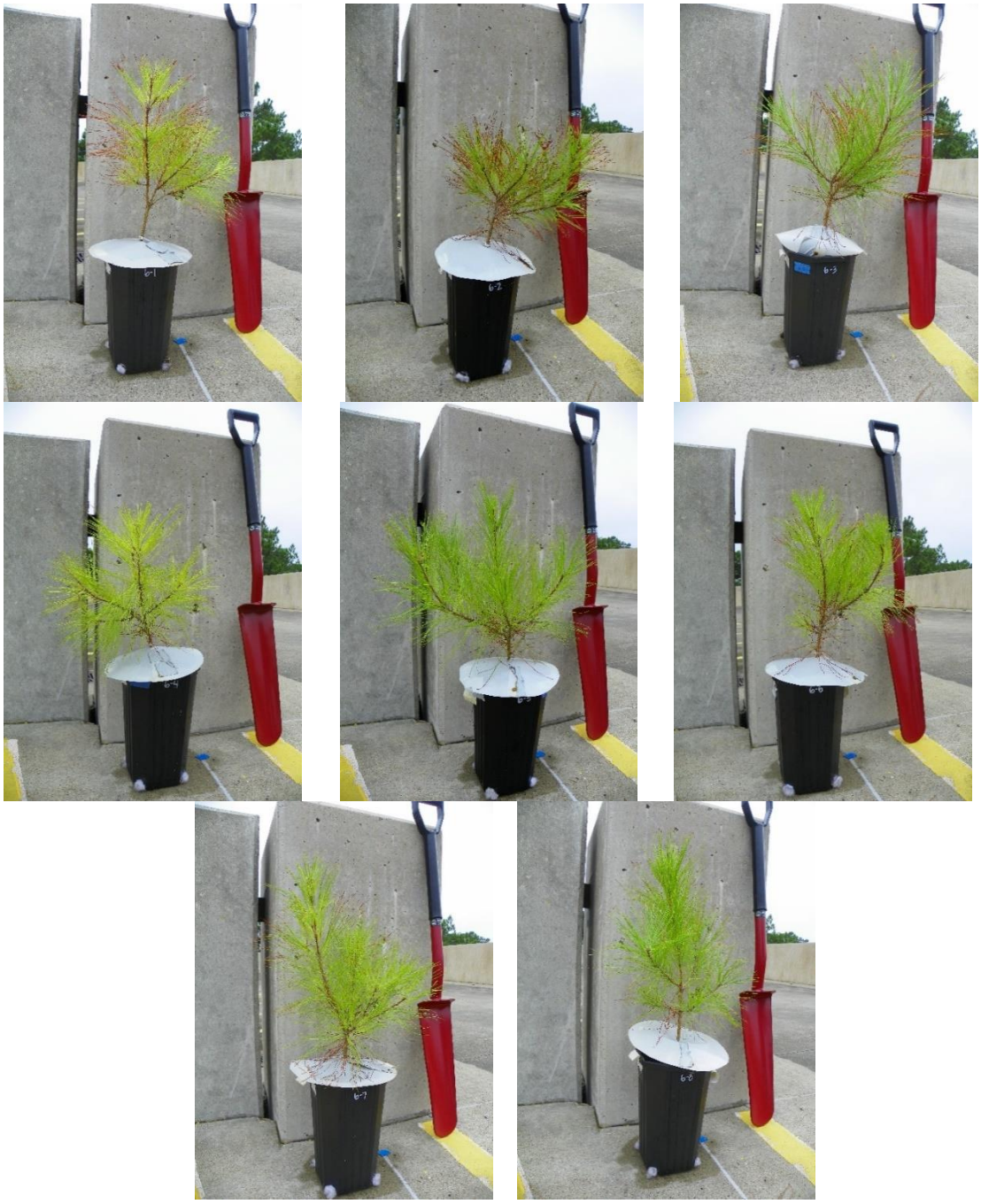

Figure 48. a-h. Photographs of 1250 mg $kg^{-1}$ treatment after one growing season, replications one through eight.

Figure 49. a-f. Representative sample from dried and ground needles. ((first value; (1) Control, (2) 250 mg $kg^{-1}$, (3) 500 mg $kg^{-1}$, (4) 750 mg $kg^{-1}$, (5) 1000 mg $kg^{-1}$, and (6) 1250 mg $kg^{-1}$) and the replication (the second value)).

# APPENDIX C

## Data Collected

Table 37.a. Complete measured data set after one growing season.

| Trt | Rep | RCDI (mm) | RCDF (mm) | HtI (cm) | HtF (cm) | Needle (cm) | Branch (cm) | pH |
|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 4.18 | 7.93 | 25.5 | 37 | 13.16 | 14.50 | 6.03 |
| 0 | 2 | 4.02 | 8.22 | 25.3 | 41 | 13.23 | 16.25 | 5.54 |
| 0 | 3 | 5.29 | 5.48 | 27.7 | 41 | 12.45 | 14.80 | 5.25 |
| 0 | 4 | 7.89 | 10.74 | 28.4 | 39 | 10.96 | 15.00 | 5.32 |
| 0 | 5 | 4.27 | 8.08 | 27.1 | 47 | 13.35 | 17.67 | 5.48 |
| 0 | 6 | 6.68 | 9.30 | 28.6 | 43 | 9.88 | 13.20 | 5.52 |
| 0 | 7 | 6.89 | 9.70 | 26.2 | 45 | 13.64 | 13.20 | 5.57 |
| 0 | 8 | 4.66 | 8.09 | 27.4 | 41 | 14.19 | 15.60 | 5.59 |
| 250 | 1 | 5.99 | 9.32 | 25.9 | 40 | 12.37 | 17.83 | 5.15 |
| 250 | 2 | 4.79 | 8.77 | 25.6 | 52 | 14.58 | 20.25 | 5.49 |
| 250 | 3 | 5.45 | 9.78 | 28.1 | 52 | 14.88 | 16.00 | 5.41 |
| 250 | 4 | 4.90 | 8.83 | 27.0 | 43 | 15.38 | 18.67 | 5.63 |
| 250 | 5 | 4.10 | 7.65 | 26.9 | 47 | 13.45 | 11.83 | 5.52 |
| 250 | 6 | 3.56 | 8.96 | 27.3 | 50 | 15.47 | 21.25 | 5.67 |
| 250 | 7 | 4.30 | 8.89 | 26.0 | 45 | 16.88 | 21.67 | 6.13 |
| 250 | 8 | 4.97 | 8.54 | 29.1 | 41 | 13.36 | 19.00 | 5.33 |
| 500 | 1 | 5.03 | 9.31 | 24.2 | 39 | 12.56 | 15.00 | 5.34 |
| 500 | 2 | 7.38 | 10.13 | 28.6 | 45 | 11.20 | 16.83 | 5.49 |
| 500 | 3 | 6.55 | 8.69 | 27.3 | 44 | 11.44 | 14.67 | 5.19 |
| 500 | 4 | 6.67 | 10.20 | 26.9 | 47 | 14.20 | 18.33 | 5.00 |
| 500 | 5 | 4.60 | 9.75 | 22.7 | 51 | 13.73 | 24.00 | 5.28 |
| 500 | 6 | 5.19 | 8.66 | 27.3 | 41 | 11.23 | 18.40 | 5.32 |
| 500 | 7 | 4.51 | 9.36 | 28.4 | 50 | 13.65 | 23.50 | 5.33 |
| 500 | 8 | 6.29 | 9.87 | 26.6 | 45 | 13.22 | 17.75 | 5.23 |
| 750 | 1 | 5.69 | 8.94 | 28.8 | 48 | 12.21 | 15.50 | 5.55 |
| 750 | 2 | 5.67 | 9.08 | 28.3 | 55 | 15.14 | 25.67 | 5.39 |
| 750 | 3 | 5.24 | 9.27 | 30.2 | 49 | 15.82 | 18.67 | 5.37 |
| 750 | 4 | 4.47 | 7.93 | 22.3 | 51 | 12.31 | 19.83 | 5.30 |
| 750 | 5 | 3.82 | 9.35 | 27.5 | 55 | 16.04 | 22.75 | 4.88 |
| 750 | 6 | 4.76 | 9.71 | 24.4 | 45 | 13.21 | 18.00 | 5.89 |
| 750 | 7 | 3.96 | 9.60 | 24.7 | 51 | 15.29 | 16.00 | 5.34 |
| 750 | 8 | 4.55 | 8.34 | 23.5 | 40 | 12.49 | 17.83 | 5.55 |
| 1000 | 1 | 4.19 | 8.95 | 26.8 | 49 | 16.34 | 21.67 | 5.13 |
| 1000 | 2 | 5.15 | 9.62 | 28.9 | 51 | 15.77 | 19.80 | 5.38 |
| 1000 | 3 | 5.42 | 10.48 | 26.4 | 50 | 14.38 | 16.57 | 5.14 |
| 1000 | 4 | 4.91 | 9.94 | 24.0 | 53 | 16.06 | 21.75 | 5.44 |
| 1000 | 5 | 4.56 | 9.94 | 29.2 | 53 | 14.76 | 21.60 | 5.86 |
| 1000 | 6 | 4.75 | 7.55 | 29.4 | 50 | 12.55 | 13.33 | 5.70 |
| 1000 | 7 | 3.93 | 9.69 | 23.6 | 45 | 14.25 | 26.67 | 5.27 |
| 1000 | 8 | 4.48 | 10.49 | 26.7 | 46 | 14.92 | 21.25 | 5.76 |
| 1250 | 1 | 4.35 | 8.64 | 26.0 | 51 | 14.54 | 20.00 | 5.44 |
| 1250 | 2 | 4.95 | 8.68 | 26.2 | 37 | 13.95 | 17.67 | 5.26 |
| 1250 | 3 | 4.62 | 9.38 | 27.4 | 45 | 15.61 | 20.00 | 5.29 |
| 1250 | 4 | 4.21 | 8.56 | 25.3 | 45 | 16.13 | 18.50 | 5.29 |
| 1250 | 5 | 4.45 | 9.71 | 23.9 | 50 | 13.71 | 24.00 | 5.32 |
| 1250 | 6 | 4.49 | 10.07 | 26.9 | 48 | 14.04 | 24.00 | 6.03 |
| 1250 | 7 | 4.62 | 9.90 | 25.7 | 56 | 15.70 | 15.67 | 5.40 |
| 1250 | 8 | 4.40 | 9.15 | 26.4 | 56 | 13.69 | 18.33 | 5.44 |

Table 37.b. Complete measured data set after one growing season (cont.)

| Trt | Rep | SQI | SQF | RS | EMRT | DQI | SDM (g) | RDM (g) | TDM (g) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 6.10 | 4.67 | 0.51 | 7749.81 | 4.73 | 20.73 | 10.58 | 31.31 |
| 0 | 2 | 6.29 | 4.99 | 0.54 | 22300.31 | 4.60 | 20.46 | 11.02 | 31.48 |
| 0 | 3 | 5.24 | 7.48 | 0.73 | 20971.00 | 3.54 | 18.64 | 13.68 | 32.32 |
| 0 | 4 | 3.60 | 3.63 | 0.48 | 12397.62 | 7.57 | 29.25 | 14.04 | 43.29 |
| 0 | 5 | 6.35 | 5.82 | 0.67 | 18208.51 | 4.41 | 19.38 | 12.90 | 32.28 |
| 0 | 6 | 4.28 | 4.63 | 0.58 | 20382.42 | 5.91 | 23.69 | 13.81 | 37.50 |
| 0 | 7 | 3.80 | 4.64 | 0.63 | 21466.67 | 5.62 | 21.58 | 13.51 | 35.09 |
| 0 | 8 | 5.88 | 5.07 | 0.65 | 14851.20 | 5.32 | 21.20 | 13.88 | 35.08 |
| 250 | 1 | 4.32 | 4.29 | 0.67 | 31994.48 | 6.74 | 23.40 | 15.64 | 39.04 |
| 250 | 2 | 5.34 | 5.93 | 0.44 | 14903.57 | 4.59 | 26.20 | 11.47 | 37.67 |
| 250 | 3 | 5.16 | 5.32 | 0.67 | 24398.75 | 5.64 | 22.92 | 15.45 | 38.37 |
| 250 | 4 | 5.51 | 4.87 | 0.63 | 22776.00 | 6.21 | 24.58 | 15.53 | 40.11 |
| 250 | 5 | 6.56 | 6.15 | 0.44 | 12464.67 | 4.10 | 24.01 | 10.56 | 34.57 |
| 250 | 6 | 7.67 | 5.58 | 0.46 | 19113.15 | 4.92 | 26.19 | 11.99 | 38.18 |
| 250 | 7 | 6.05 | 5.06 | 0.54 | 25719.27 | 5.96 | 26.76 | 14.44 | 41.20 |
| 250 | 8 | 5.86 | 4.80 | 0.59 | 13315.90 | 5.23 | 21.42 | 12.57 | 33.99 |
| 500 | 1 | 4.81 | 4.19 | 0.53 | 8502.96 | 6.12 | 24.20 | 12.93 | 37.13 |
| 500 | 2 | 3.88 | 4.44 | 0.46 | 27381.77 | 7.28 | 32.90 | 15.20 | 48.10 |
| 500 | 3 | 4.17 | 5.07 | 0.47 | 7834.42 | 4.39 | 21.44 | 10.12 | 31.56 |
| 500 | 4 | 4.03 | 4.61 | 0.56 | 21729.09 | 7.50 | 30.66 | 17.24 | 47.90 |
| 500 | 5 | 4.93 | 5.23 | 0.60 | 28520.00 | 6.02 | 25.99 | 15.58 | 41.57 |
| 500 | 6 | 5.26 | 4.73 | 0.65 | 19775.70 | 7.11 | 26.90 | 17.61 | 44.51 |
| 500 | 7 | 6.30 | 5.34 | 0.52 | 12630.09 | 5.82 | 27.83 | 14.48 | 42.31 |
| 500 | 8 | 4.23 | 4.56 | 0.53 | 16019.80 | 6.08 | 25.46 | 13.62 | 39.08 |
| 750 | 1 | 5.06 | 5.37 | 0.59 | 18367.50 | 6.01 | 26.78 | 15.72 | 42.50 |
| 750 | 2 | 4.99 | 6.06 | 0.46 | 22524.60 | 5.38 | 30.25 | 14.01 | 44.26 |
| 750 | 3 | 5.76 | 5.29 | 0.57 | 18442.33 | 6.60 | 29.71 | 16.81 | 46.52 |
| 750 | 4 | 4.99 | 6.43 | 0.47 | 16996.50 | 5.68 | 32.94 | 15.61 | 48.55 |
| 750 | 5 | 7.20 | 5.89 | 0.44 | 19071.55 | 5.83 | 32.85 | 14.57 | 47.42 |
| 750 | 6 | 5.13 | 4.64 | 0.64 | 28268.00 | 8.13 | 30.82 | 19.63 | 50.45 |
| 750 | 7 | 6.24 | 5.32 | 0.34 | 16013.82 | 5.38 | 33.06 | 11.30 | 44.36 |
| 750 | 8 | 5.16 | 4.80 | 0.55 | 26661.14 | 6.22 | 26.45 | 14.64 | 41.09 |
| 1000 | 1 | 6.40 | 5.47 | 0.63 | 17644.35 | 7.46 | 32.31 | 20.37 | 52.68 |
| 1000 | 2 | 5.61 | 5.30 | 0.52 | 22072.50 | 7.59 | 36.13 | 18.73 | 54.86 |
| 1000 | 3 | 4.87 | 4.77 | 0.66 | 42406.36 | 9.75 | 36.95 | 24.35 | 61.30 |
| 1000 | 4 | 4.89 | 5.33 | 0.46 | 16695.35 | 7.85 | 40.12 | 18.64 | 58.76 |
| 1000 | 5 | 6.40 | 5.33 | 0.54 | 25480.00 | 8.03 | 37.48 | 20.20 | 57.68 |
| 1000 | 6 | 6.19 | 6.62 | 0.38 | 7921.52 | 3.00 | 20.11 | 7.66 | 27.77 |
| 1000 | 7 | 6.01 | 4.65 | 0.54 | 24233.33 | 7.71 | 32.36 | 17.62 | 49.98 |
| 1000 | 8 | 5.96 | 4.39 | 0.38 | 19067.57 | 6.84 | 35.03 | 13.18 | 48.21 |
| 1250 | 1 | 5.98 | 5.91 | 0.52 | 14724.39 | 4.51 | 23.20 | 12.11 | 35.31 |
| 1250 | 2 | 5.29 | 4.26 | 0.60 | 16744.20 | 6.63 | 24.50 | 14.75 | 39.25 |
| 1250 | 3 | 5.93 | 4.80 | 0.60 | 21707.64 | 7.94 | 32.08 | 19.27 | 51.35 |
| 1250 | 4 | 6.01 | 5.26 | 0.50 | 8308.41 | 5.35 | 25.87 | 12.94 | 38.81 |
| 1250 | 5 | 5.37 | 5.15 | 0.45 | 23952.83 | 7.19 | 36.44 | 16.48 | 52.92 |
| 1250 | 6 | 5.99 | 4.77 | 0.53 | 10149.32 | 6.95 | 30.11 | 16.04 | 46.15 |
| 1250 | 7 | 5.56 | 5.66 | 0.59 | 27479.69 | 7.51 | 34.90 | 20.45 | 55.35 |
| 1250 | 8 | 6.00 | 6.12 | 0.58 | 16341.27 | 6.12 | 30.38 | 17.65 | 48.03 |

Table 37.c. Complete measured data set after one growing season (cont.)

| Trt | Rep | Na | P | K | Ca | Mg | B |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 896 | 1510 | 11048 | 3146 | 1075 | 22 |
| 0 | 2 | 94 | 1603 | 10589 | 3361 | 1498 | 20 |
| 0 | 3 | 169 | 1621 | 14677 | 3712 | 1066 | 22 |
| 0 | 4 | 119 | 1854 | 15018 | 2408 | 1049 | 19 |
| 0 | 5 | 356 | 2200 | 11943 | 3255 | 1210 | 24 |
| 0 | 6 | 132 | 1320 | 11462 | 3071 | 1190 | 22 |
| 0 | 7 | 1215 | 1539 | 9216 | 2992 | 1367 | 28 |
| 0 | 8 | 73 | 1785 | 14665 | 2437 | 1036 | 24 |
| 250 | 1 | 78 | 5856 | 13928 | 5406 | 1930 | 35 |
| 250 | 2 | 137 | 4442 | 14077 | 4172 | 1767 | 30 |
| 250 | 3 | 752 | 5075 | 15606 | 4920 | 1723 | 36 |
| 250 | 4 | 236 | 7034 | 14712 | 5999 | 1844 | 36 |
| 250 | 5 | 177 | 5002 | 13505 | 4017 | 1701 | 28 |
| 250 | 6 | 335 | 6541 | 15141 | 4267 | 1703 | 27 |
| 250 | 7 | 96 | 7941 | 18801 | 5603 | 1912 | 29 |
| 250 | 8 | 691 | 8182 | 17151 | 4718 | 1402 | 33 |
| 500 | 1 | 186 | 5393 | 14248 | 4367 | 1881 | 38 |
| 500 | 2 | 874 | 7955 | 12549 | 5745 | 2222 | 31 |
| 500 | 3 | 1318 | 7050 | 16790 | 5974 | 1929 | 40 |
| 500 | 4 | 77 | 8393 | 14112 | 5794 | 1808 | 30 |
| 500 | 5 | 58 | 6009 | 15409 | 4948 | 1665 | 30 |
| 500 | 6 | 170 | 8281 | 13512 | 5018 | 1995 | 31 |
| 500 | 7 | 646 | 7277 | 14988 | 4793 | 1948 | 35 |
| 500 | 8 | 630 | 5527 | 12315 | 5175 | 2354 | 43 |
| 750 | 1 | 301 | 7325 | 13685 | 5934 | 2299 | 42 |
| 750 | 2 | 160 | 8105 | 13673 | 5147 | 2671 | 40 |
| 750 | 3 | 814 | 7795 | 12508 | 4521 | 1536 | 31 |
| 750 | 4 | 103 | 7811 | 14789 | 4710 | 1642 | 27 |
| 750 | 5 | 804 | 6637 | 13076 | 4722 | 1939 | 41 |
| 750 | 6 | 746 | 9244 | 13592 | 5234 | 2217 | 36 |
| 750 | 7 | 240 | 6673 | 11657 | 5046 | 2000 | 25 |
| 750 | 8 | 204 | 9773 | 17337 | 5387 | 2066 | 39 |
| 1000 | 1 | 183 | 7990 | 12827 | 4536 | 2080 | 32 |
| 1000 | 2 | 364 | 7649 | 13029 | 3771 | 1640 | 26 |
| 1000 | 3 | 121 | 8824 | 13725 | 5605 | 2322 | 32 |
| 1000 | 4 | 676 | 11087 | 15435 | 5437 | 2068 | 28 |
| 1000 | 5 | 378 | 10194 | 15836 | 5431 | 1986 | 31 |
| 1000 | 6 | 1435 | 8521 | 8745 | 6425 | 2478 | 45 |
| 1000 | 7 | 656 | 9456 | 15247 | 4630 | 2047 | 33 |
| 1000 | 8 | 990 | 10409 | 13596 | 5195 | 2197 | 31 |
| 1250 | 1 | 612 | 15135 | 18738 | 8526 | 2470 | 42 |
| 1250 | 2 | 221 | 11819 | 16025 | 5333 | 2276 | 37 |
| 1250 | 3 | 264 | 12644 | 16275 | 7081 | 2183 | 33 |
| 1250 | 4 | 610 | 10237 | 14666 | 4846 | 2021 | 39 |
| 1250 | 5 | 472 | 9509 | 15062 | 4122 | 1825 | 29 |
| 1250 | 6 | 508 | 10853 | 13866 | 5452 | 2227 | 41 |
| 1250 | 7 | 653 | 12307 | 15277 | 5870 | 2617 | 47 |
| 1250 | 8 | 235 | 12051 | 15926 | 6127 | 2309 | 32 |

Table 37.d. Complete measured data set after one growing season (cont.)

| Trt | Rep | Fe | Mn | Zn | Cu | S | Mn | Al | Mo | As | Ni |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 126 | 255 | 28 | 5 | 1207 | 255 | 82 | 0 | 1 | 1 |
| 0 | 2 | 69 | 228 | 67 | 5 | 1133 | 228 | 51 | 0 | 1 | 1 |
| 0 | 3 | 93 | 314 | 30 | 4 | 1294 | 314 | 59 | 0 | 1 | 1 |
| 0 | 4 | 89 | 402 | 27 | 3 | 1297 | 402 | 64 | 0 | 1 | 1 |
| 0 | 5 | 73 | 316 | 43 | 3 | 1310 | 316 | 60 | 0 | 1 | 1 |
| 0 | 6 | 64 | 327 | 28 | 3 | 1091 | 327 | 56 | 0 | 1 | 1 |
| 0 | 7 | 63 | 276 | 33 | 11 | 1354 | 276 | 44 | 0 | 2 | 1 |
| 0 | 8 | 56 | 265 | 23 | 4 | 1319 | 265 | 32 | 0 | 2 | 1 |
| 250 | 1 | 82 | 564 | 68 | 4 | 1721 | 564 | 75 | 0 | 1 | 1 |
| 250 | 2 | 94 | 418 | 57 | 3 | 1409 | 418 | 42 | 0 | 1 | 1 |
| 250 | 3 | 115 | 377 | 64 | 3 | 1424 | 377 | 54 | 0 | 2 | 1 |
| 250 | 4 | 91 | 414 | 51 | 3 | 1178 | 414 | 32 | 0 | 2 | 1 |
| 250 | 5 | 63 | 289 | 57 | 4 | 1294 | 289 | 28 | 0 | 1 | 1 |
| 250 | 6 | 114 | 286 | 37 | 4 | 1398 | 286 | 32 | 0 | 1 | 1 |
| 250 | 7 | 74 | 402 | 59 | 8 | 1680 | 402 | 35 | 0 | 1 | 1 |
| 250 | 8 | 136 | 405 | 76 | 10 | 1577 | 405 | 66 | 0 | 2 | 1 |
| 500 | 1 | 70 | 451 | 48 | 3 | 1078 | 451 | 58 | 0 | 2 | 1 |
| 500 | 2 | 80 | 449 | 71 | 6 | 1119 | 449 | 44 | 0 | 2 | 1 |
| 500 | 3 | 60 | 478 | 70 | 3 | 1375 | 478 | 61 | 0 | 2 | 1 |
| 500 | 4 | 65 | 507 | 51 | 6 | 1272 | 507 | 44 | 0 | 2 | 1 |
| 500 | 5 | 52 | 536 | 97 | 4 | 1295 | 536 | 43 | 0 | 1 | 1 |
| 500 | 6 | 72 | 602 | 76 | 4 | 1343 | 602 | 58 | 0 | 1 | 1 |
| 500 | 7 | 101 | 598 | 72 | 5 | 1185 | 598 | 74 | 0 | 2 | 1 |
| 500 | 8 | 56 | 575 | 66 | 4 | 1333 | 575 | 52 | 0 | 2 | 1 |
| 750 | 1 | 64 | 603 | 63 | 3 | 1337 | 603 | 50 | 0 | 2 | 1 |
| 750 | 2 | 68 | 611 | 70 | 3 | 1406 | 611 | 45 | 0 | 2 | 1 |
| 750 | 3 | 58 | 642 | 81 | 4 | 1034 | 642 | 35 | 0 | 2 | 1 |
| 750 | 4 | 82 | 534 | 64 | 4 | 1122 | 534 | 42 | 0 | 1 | 1 |
| 750 | 5 | 53 | 623 | 48 | 3 | 1245 | 623 | 29 | 0 | 2 | 1 |
| 750 | 6 | 58 | 609 | 70 | 4 | 1587 | 609 | 26 | 0 | 2 | 1 |
| 750 | 7 | 62 | 540 | 58 | 3 | 998 | 540 | 27 | 0 | 2 | 1 |
| 750 | 8 | 50 | 562 | 90 | 3 | 1391 | 562 | 25 | 0 | 2 | 1 |
| 1000 | 1 | 42 | 852 | 90 | 4 | 1171 | 852 | 26 | 0 | 1 | 1 |
| 1000 | 2 | 54 | 620 | 62 | 3 | 957 | 620 | 26 | 0 | 1 | 1 |
| 1000 | 3 | 54 | 853 | 92 | 3 | 1435 | 853 | 29 | 0 | 2 | 1 |
| 1000 | 4 | 56 | 641 | 59 | 4 | 1214 | 641 | 27 | 0 | 2 | 1 |
| 1000 | 5 | 53 | 678 | 69 | 3 | 1278 | 678 | 29 | 0 | 2 | 1 |
| 1000 | 6 | 115 | 990 | 65 | 3 | 1146 | 990 | 64 | 0 | 4 | 1 |
| 1000 | 7 | 45 | 683 | 58 | 2 | 1178 | 683 | 22 | 0 | 1 | 1 |
| 1000 | 8 | 161 | 769 | 62 | 2 | 1475 | 769 | 33 | 0 | 2 | 1 |
| 1250 | 1 | 48 | 977 | 69 | 3 | 1638 | 977 | 26 | 0 | 2 | 1 |
| 1250 | 2 | 49 | 879 | 76 | 3 | 1361 | 879 | 27 | 0 | 2 | 1 |
| 1250 | 3 | 46 | 885 | 72 | 4 | 1334 | 885 | 26 | 0 | 2 | 1 |
| 1250 | 4 | 50 | 684 | 48 | 2 | 958 | 684 | 28 | 0 | 2 | 1 |
| 1250 | 5 | 91 | 648 | 66 | 4 | 1185 | 648 | 62 | 0 | 2 | 1 |
| 1250 | 6 | 65 | 864 | 61 | 3 | 1240 | 864 | 26 | 0 | 2 | 1 |
| 1250 | 7 | 43 | 1063 | 67 | 3 | 1710 | 1063 | 29 | 0 | 2 | 1 |
| 1250 | 8 | 72 | 753 | 38 | 2 | 1345 | 753 | 24 | 0 | 2 | 1 |

Table 37.e. Complete measured data set after one growing season (cont.)

| Trt | Rep | Chlorosis | Necrosis | MunsellColorL | MunsellColorG |
|---|---|---|---|---|---|
| 0 | 1 | 0 | 0 | 5GY 6/6 | 5Y 5/4 |
| 0 | 2 | 0 | 0 | 5GY 5/6 | 5Y 5/6 |
| 0 | 3 | 0 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 0 | 4 | 0 | 0 | 5GY 5/6 | 5Y 6/4 |
| 0 | 5 | 0 | 0 | 5GY 5/6 | 2.5Y 6/4 |
| 0 | 6 | 0 | 0 | 5GY 6/6 | 2.5Y 6/6 |
| 0 | 7 | 0 | 0 | 5GY 5/6 | 2.5Y 6/4 |
| 0 | 8 | 0 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 250 | 1 | 0 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 250 | 2 | 0 | 0 | 5GY 6/6 | 5Y 6/5 |
| 250 | 3 | 0 | 0 | 5GY 6/6 | 5Y 7/4 |
| 250 | 4 | 0 | 0 | 5GY 6/6 | 5Y 6/4 |
| 250 | 5 | 0 | 0 | 5GY 5/6 | 5Y 6/5 |
| 250 | 6 | 0 | 0 | 5GY 6/6 | 5Y 6/4 |
| 250 | 7 | 0 | 0 | 5GY 5/6 | 5Y 6/5 |
| 250 | 8 | 0 | 0 | 5GY 6/6 | 5Y 6/4 |
| 500 | 1 | 1 | 0 | 2.5GY 7/6 | 2.5Y 6/4 |
| 500 | 2 | 0 | 0 | 5GY 5/6 | 2.5Y 6/4 |
| 500 | 3 | 1 | 0 | 2.5GY 6/6 | 2.5Y 6/6 |
| 500 | 4 | 0 | 1 | 5GY 5/6 | 2.5Y 6/6 |
| 500 | 5 | 0 | 0 | 5GY 5/6 | 5Y 6/4 |
| 500 | 6 | 1 | 0 | 2.5GY 5/6 | 2.5Y 6/4 |
| 500 | 7 | 1 | 0 | 2.5GY 6/6 | 2.5Y 6/6 |
| 500 | 8 | 1 | 0 | 5GY 6/6 | 5Y 6/4 |
| 750 | 1 | 1 | 0 | 5GY 7/8 | 2.5Y 6/4 |
| 750 | 2 | 1 | 0 | 5GY 5/6 | 2.5Y 6/6 |
| 750 | 3 | 1 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 750 | 4 | 0 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 750 | 5 | 1 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 750 | 6 | 0 | 1 | 5GY 5/6 | 2.5Y 5/6 |
| 750 | 7 | 1 | 1 | 2.5GY 6/8 | 2.5Y 6/4 |
| 750 | 8 | 1 | 0 | 5GY 6/6 | 2.5Y 5/6 |
| 1000 | 1 | 1 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 1000 | 2 | 1 | 0 | 5GY 6/6 | 2.5Y 6/4 |
| 1000 | 3 | 1 | 1 | 5GY 6/6 | 2.5Y 7/4 |
| 1000 | 4 | 1 | 0 | 5GY 5/6 | 2.5Y 7/4 |
| 1000 | 5 | 1 | 1 | 2.5GY 5/8 | 2.5Y 6/4 |
| 1000 | 6 | 1 | 1 | 2.5GY 7/8 | 2.5Y 6/4 |
| 1000 | 7 | 1 | 0 | 5GY 6/6 | 2.5Y 7/6 |
| 1000 | 8 | 1 | 1 | 5GY 7/8 | 2.5Y 6/6 |
| 1250 | 1 | 1 | 1 | 2.5GY 6/8 | 2.5Y 6/6 |
| 1250 | 2 | 1 | 1 | 5GY 6/6 | 2.5Y 6/6 |
| 1250 | 3 | 1 | 1 | 5GY 5/6 | 2.5Y 7/6 |
| 1250 | 4 | 1 | 1 | 2.5GY 8/8 | 2.5Y 7/6 |
| 1250 | 5 | 1 | 1 | 5GY 6/6 | 2.5Y 6/4 |
| 1250 | 6 | 1 | 1 | 2.5GY 6/8 | 2.5Y 6/6 |
| 1250 | 7 | 1 | 1 | 5GY 6/6 | 2.5Y 7/6 |
| 1250 | 8 | 1 | 1 | 5GY 6/6 | 2.5Y 6/4 |

# VITA

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