ABSTRACT

PETTIT, AARON DAVID. Nutrient Management of Horticulture Crops: The Restriction of *Vitis vinifera* Growth in Acid Sandy Soil and Nitrogen Loading in Container Grown *Tilia cordata, Acer rubrum,* and *Cedrus deodara*. (Under the direction of Dr. John L. Havlin).

The majority of NC agricultural lands are characterized as acid soils that are highly weathered which can be of great concern in establishment and management of *V. vinifera* vineyards. This study evaluated soil acidity effects on vine shoot and root growth. Soil with target pH treatments of 4, 5, 6, and 7 were established in a Norfolk sandy loam by adding lime ($CaCO_3$). There was a significant improvement in plant growth as target soil pH increased from 4 to 5; the response was less distinguishable as target soil pH increased from 5 to 7. As soil pH increased, both tissue and exchangeable soil aluminum (Al) were greatly reduced. The Al response was most evident as target soil pH increased from 4 to 5. There were significant changes in soil exchangeable and plant tissue nutrient concentrations as soil pH increased, but this was not seen with every nutrient and varied with soil pH and tissue (leaf, stem, and root). There was also a notable antagonistic effect on magnesium (Mg) and potassium (K) uptake from increasing calcium (Ca) concentration in the soil. Results illustrate soil pH can have an impact on plant growth and nutrient uptake in *V. vinifera*.

Adequate nitrogen (N) concentration within container nursery trees has always been a concern for high-quality growth and development. Nitrogen demand can vary greatly with tree species, age, growth media, and climate. The objective of this study was to evaluate three commonly grown container tree species and their ability to assimilate N with increasing slow-release N rates. *Tilia cordata, Acer rubrum,* and *Cedrus deodara* were grown in pine bark nursery media at 3, 9, 18, and 27 g pot^{-1} of slow-release N. Tree species varied in growth response to the uptake and distribution of N. *Cedrus deodara* was a low N user, with little or no changes in growth or tissue N concentration. *Tilia cordata* and *Acer rubrum* both were considered high N users. Increasing N rate increased N concentration and N use efficiency in *Tilia cordata*, and increased total growth in *Acer rubrum*. Our conclusion was to fertilize each of these three tree species with sufficient N in the nursery environment to

maintain vigor and adaptability during the stress of transplanting into a landscape or forestry site.

Nutrient Management of Horticulture Crops: The Restriction of *Vitis vinifera* Growth in Acid Sandy Soil and Nitrogen Loading in Container Grown *Tilia cordata, Acer rubrum*, and *Cedrus deodara*

by
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DEDICATION

To my Mom (Dawn Pettit) and Grandpa (David McLelland).

BIOGRAPHY

I grew up in a small city called Statesville, located in the Piedmont of North Carolina. When I was a little boy, I always remember work in the garden with my grandpa. My interest peeked in plant science and soil fertility while attending agriculture and horticulture classes at North Iredell High School and also as a field and greenhouse worker at a vegetable farm called Harvest Heights. After having back surgery at the age of twenty, I decided to go back to school and chose the field of horticulture at Wilkes Community College. After community college I decided I would like to obtain a bachelor's degree. I moved to Raleigh and began working at North Carolina State University (NCSU) in June, 2004; and started a bachelor's program in horticulture in January, 2005. I finished my Bachelor of Science degree in May 2009 while working full-time as a horticulture specialist for NCSU Grounds Management. Afterwards I started graduate school, I had a few hardships that set me back, but with the help of my advisor I was able to pull through. I started a new job at NCDA&CS as an agronomist in November 2012.

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CHAPTER 1

The Restriction of *Vitis vinifera* Growth in an Acid Sandy Soil

Abstract

Vitis vinifera is an important grape species in the wine industry. One obstacle for *V. vinifera* production in North Carolina (NC) is the highly weathered acid soils. The objective of this study was to evaluate the effect of increasing soil acidity on vine shoot and root growth. *V. vinifera* plants were grown in containers of Norfolk loamy sand at target pH 4, 5, 6, and 7. As target soil pH increased from 4 to 5, an evident improvement in plant growth was observed. Above target pH 5 there was little or no plant growth response. As soil pH increased, Al levels in the tissue, as well as exchangeable Al in the soil, were greatly reduced. There were significant changes in soil exchangeable and plant tissue nutrients as soil pH increased, but this was not seen with every nutrient and varied with soil pH and tissue (leaf, stem, and root). There were also notable antagonistic effects from increasing Ca concentrations on Mg and K uptake. Results demonstrated increasing soil pH can have lead to significant improvement in *V. vinifera* growth and nutrient uptake.

Introduction

In North Carolina (NC) the wine grape industry has expanded in the past decade, with over 110 wineries and 400 vineyards in the state, ranking 9th in wine and 10th in grape production in the U.S. The majority of vineyards located in the Piedmont (NCDC, 2010). The USDA estimated approximately 1800 acres of grape production in NC, producing an estimated 5200 tons of grapes (USDA, 2012). In 2009, the NC wine industry had an estimated economic impact of $1.28 billion, an increase of 38% since 2005 (Rimerman, 2009).

There are four species of grapes grown in NC: *Vitis vinifera*, *V. labruscana*, *V. aesrivalis*, and *V. rotundifolia* (Muscadine) (Poling, 2013). Wine grape species and varieties greatly influence wine quality along with other factors. The European species *V. vinifera* is one of the most popular wine grapes species, accounting for over 90% of the wine grape

production in the world (Haapala, 2004). The native species *V. labruscana* and *V. rotundifolia* are also well known for wine production in the United States (Weaver, 1976; Haapala, 2004). Compared to the commonly grown *V. rotundifolia, V. vinifera* is an increasingly popular species in the Piedmont and Mountain regions of NC (Carroll et al., 1991; Poling, 2013). The most common *V. vinifera* varieties are Chardonnay (white) and Cabernet Sauvignon (red), which are the most cold-hardy and disease-resistant varieties (Reisch et al., 1993; NC Cooperative Extension, 2012; Poling, 2013). The major problems with *V. vinifera* production in the eastern U.S. are sun-scorching of leaves and berries, diseases (Weaver, 1976), and poor establishment and growth in acid soil (Himelrick, 1991). Acid soils can inhibit root growth of *V. vinifera* leading to shallow root systems which ultimately results in decreased water and nutrient availability (Kirchhof et al, 1991).

North Carolina soils are predominantly Ultisols, which are generally highly weathered acid soils with low base saturation (BS) that limit plant growth and yield (Gonzalez-Erico et al., 1979; Wright, 1989; Buol et al. 2003). Most Piedmont NC soils were formed from acid igneous parent material containing large amounts of silicon (Si) and aluminum (Al), but contain relatively small amounts of phosphorus (P), calcium (Ca), and magnesium (Mg) (Buol, 1999). Factors that affect acidification of soils are high precipitation and leaching of basic cations, plant exudates, decay of plant and animal residues, crop removal of basic cations, use of N fertilizers, and inputs of acids from atmospheric sources (H_2CO_3, H_2SO_4 and HNO_3) (Stephen and Powers, 1949; Whitney and Lamond, 1993). Soil pH and exchangeable acidity can be influenced by soil mineralogy, organic ligands, and Al and iron (Fe) hydrolysis (Thomas, 1988; Whitney and Lamond, 1993). Acid inputs and leaching cause minerals to dissolve resulting in the release of cations (Al^{+3}, Mn^{+2} and Fe^{3+}), which either hydrolyze to form H^+ or replace basic cations on the exchange complex. Displaced basic cations can then be taken up by plants or leached, thereby decreasing the base saturation of the soil (Hodges, 2010). Acidity in soils comes mainly from two cations, Al^{+3} and H^+, where Al^{+3} represents the majority of exchangeable acid in mineral soils and H^+ in organic soils (Thomas, 1988).

Excessive Al associated with low pH in mineral soils is a major limiting factor to plant growth (Wright, 1989; Mossor-Pietraszewska; 2001). In soil, Al occurs as amorphous

and layered aluminosilicate clay minerals, exchangeable Al^{+3}, and solution Al^{+3}. The concentration of soluble Al^{+3} depends on soil pH, the amount and type of primary and secondary Al containing minerals, Al^{+3} adsorbed to inorganic surfaces, and binding of Al with organic complexes (Wright, 1989). In soil with pH $\leq$ 5, exchangeable Al^{+3} buffers soluble Al^{+3} and pH. At pH > 5.5 exchangeable Al^{+3} is generally < 10% of CEC, while $Al(OH)^{+2}$ and $Al(OH)_2^{+}$ forms dominate the soil solution. The species $Al(OH)_3$ and $Al(OH)_4^{-}$ are the dominate forms at pH $\geq$ 7 (Gonzalez-Erico et al., 1979; Thomas and Hargrove, 1984). Relative toxicity for most plants, Al^{+3} is generally more toxic than hydrolyzed Al species (Parker et al, 1987; Thomas, 1988; Wright 1989; Vardar and Unal, 2007).

Under conditions of low pH and elevated soluble Al^{+3}, grape vine growth can be reduced. The most common symptom of Al toxicity is restricted root growth caused by inhibition of cell division and elongation in root tips, which creates a root system that lacks a normal branch structure (Foy et al., 1978; Wright, 1989; Vardar and Unal, 2007; Inostroza-Blancheteau et al., 2008). Al toxicity at the root tips reduces water and nutrient uptake (Kirchhof et al., 1991). Shoot growth is adversely affected due to increased resistance in translocation of water, reduced stomatal aperture, and decreased photosynthetic activity resulting in chlorosis and necrosis of leaves, decreased leaf number and size, and decreased shoot biomass (Thornton et al., 1986).

Low nutrient uptake associated with Al toxicity results in poor plant growth in acid soils. Phosphorus deficiency symptoms are common in plants exhibiting Al toxicity. Solubility and subsequent availability of P is reduced by precipitation as Al/Fe P compounds (Schulte, 1992; Havlin et al., 2005; Wong, 2005; Inostroza-Blancheteau et al., 2008). High levels of Al can also lead to Ca, Mg, potassium (K), and molybdenum (Mo) deficiencies, due to Al interference with uptake, transport, and utilization of these nutrients (Foy et al., 1978; Wright, 1989; Schulte, 1992; Mossor-Pietraszewska; 2001). For example, in the leaves of *Acer saccharum* grown in solution culture of pH 4 with elevated Al, the levels of P, K, Ca, and Mg were reduced by 66%, 16%, 17%, and 50%, respectively (Thornton et al., 1986). Increased Al concentration has been shown to reduce NO_3^{-} uptake in some plants (Rout et al., 2001). Low pH can also increase solubility of the micronutrients Fe, manganese (Mn), copper (Cu), and zinc (Zn), leading to potential toxicity problems. These toxicities can occur

with or without Al toxicity in acid soils (Evans and Kamprath, 1970; Thomas, 1988; Withers, 1993; Hodges, 2010).

A soil pH of 5.5 has been suggested as a critical level in the eastern U.S. for *V. vinifera* (Bates and Wolf, 2009). Other species such as *V. labruscana* are well adapted to soils with pH < 5.5 (Himelick, 1991; Bates et al, 2002). *V. labruscana* (Concord grapevine) cultivars grown in soil of pH 5 had no significant loss of vegetative growth (Bates et al, 2002), while field observations showed *V. labruscana* cultivars had higher levels of Al in the leaves and greater growth under acid soil condition compared to *V. vinifera* cultivars grown under the same conditions (Himelrick, 1991). A study in South Africa showed that *V. vinifera* fruit yield decreased as soil pH increased, where plants grown at soil pH 7 exhibited the lowest yield even though biomass increased with increasing soil pH (Wooldridge et al, 2010). In the same study, wine quality was determined to be better in lower pH soil versus higher pH soils.

In media culture, grape cultivars 'Norton' and 'Vidal blanc' were grown at 4.5, 5.9, 7.2, and 8.5 pH (Kering and Kaps, 2011). Plants grown at pH 4.5 had reduced growth and showed an increase in nutrient deficiency symptoms; however, at pH 5.9 no visual symptoms were observed. The 'Norton' variety showed nutrient deficiency symptoms at the end of the experiment at ≥ pH 7.2; whereas 'Vidal blanc' did well at all pH levels except 4.5 (Kering and Kaps, 2011). Studies have also shown Al tissue concentrations increase with decreasing pH (Himelrick, 1991; Kering and Kaps, 2011).

Management of low pH vineyard soils can be achieved by two methods: increasing soil pH to optimum levels by addition of lime or using cultivars or rootstocks tolerant of low pH. First, lime neutralizes soluble and exchangeable acid by replacement of Al^{+3} and H^{+} on exchange sites with Ca^{+2} and /or Mg^{+2} depending on lime source (Withers, 1993). Once in the soil, the anion in the lime material hydrolyzes to form hydroxide which combines with H^{+} to form water or combines with Al to form insoluble hydoxylated Al (Hede et al., 2001; Hodges, 2010). Other benefits of lime include increased nutrient availability, increased N_2 fixation in legumes, improved soil structure, and reduced incidence of some pathogens (Withers, 1993).

The reaction rate of lime in reducing soil acidity is influenced by particle size (fineness), chemical composition ($CaCO_3$, $MgCO_3$, CaO, and $Ca(OH)_2$), and physical characteristics (hardness) of the material (Withers, 1993). The finer the particle size of the lime material, the greater the surface area in contact with water and soil which increases the reaction rate as compared to course particles (Withers, 1993). Incorporation of surface-applied lime increases the depth of the acid neutralized due to its low water solubility and mobility in soil (Gonzalez-Erico et al., 1979). For example, inhibition of grape root growth in acid subsoil was overcome by incorporating lime into the subsoil (Kirchhof et al, 1991). Establishing optimum soil pH is critical for optimum growth and yield in the vineyard.

The second method of alleviating stress of low pH and Al toxicity is by use of plants tolerant of acid soil conditions. This is usually accomplished by selection of using cultivars or rootstocks with known tolerance to Al toxicity. Grafting desirable scion wood onto rootstock is a common practice in the fruit industry for disease resistance, vigor control, and soil adaptability. Al tolerant rootstocks are available to help overcome Al toxicity in *V. vinifera*. Himelrick (1991) observed SO4 and 3309 rootstocks, as well as native varieties of *V. labruscana* 'Concord' and 'Catawba', were tolerant to acid soils as compared to several other rootstocks and non-grafted *V. vinifera*. Another study of five rootstocks grafted to *V. vinifera* 'Pinot Noir' showed that SO4 rootstock produced the highest yield and shoot growth under acid soil conditions (pH 5), while the lowest yield was observed with Riparia rootstock (Vasconcelos, 2001). Under extreme acid soil conditions (pH 4) 100R, Freedom, 44-53M, and SO4 rootstocks were the most tolerant; however rootstock and grape growth parameters changed greatly as soil pH increased (Taylor et al., 2001). Over three pH ranges (4, 5, 6) Couderc 3309, Kober 125AA, and Freedom were ranked the highest among the five growth parameters (Taylor et al., 2001). A commonly used rootstock in NC, 101-14, ranked 11^{th} overall and 19^{th} at pH of 4, with improved performance as soil pH increased to 6 (Taylor et al., 2001).

Mechanisms associated with Al tolerance include exclusion of Al from the root apex or internal detoxification of Al in the root. Exclusion is achieved in some plant species through the excretion of chelating acids which form a strong bond with solution Al^{+3}, reducing Al^{+3} uptake by the plant. Citrate, oxalate, and malate are common examples of

chelates excreted by roots (Barcelo and Poschenrider, 2002; Vardar and Unal, 2007; Inostroza-Blancheteau et al., 2008). Another exclusion process is alkalization of the root rhizosphere by the removal or reduction of H^+ through ion uptake, thus raising rhizosphere pH to decrease Al solubility (Foy et al., 1978; Schulze et al, 2005). The form of N (NO_3^- or NH_4^+) plays a major role in rhizosphere pH, with NH_4^+ uptake resulting in H^+ flux out of the roots to lower soil pH and increase soil exchangeable Al^{+3}; the opposite is true for NO_3^- uptake which results in the release of OH^- and HCO_3^- whereby soil pH around the rhizosphere increases (Tan, 1992; Rout et al. 2001).

The detoxification or accumulation mechanisms refer to the formation of Al stable, less toxic compounds in the plant root; compounds are stored in vacuoles or other cell components depending on the species. For example, *Hydrangea macrophylla* sepals turn from pink to blue under increased Al^{+3} concentration. Other plants have the ability for Al accumulation in the leaves (≥ 1% of dry weight) (Foy et al., 1978; Vardar and Unal, 2007; Inostroza-Blancheteau et al., 2008). Also, Al transport into the cytoplasm where pH is 7 would lower soluble Al (Inostroza-Blancheteau et al., 2008). The more acid tolerant *V. labruscana* had higher levels of Al within leaf tissue, which indicates the mechanism of detoxification.

The research emphasis in NC has been primarily with muscadine grapes (*V. rotundifolia*), with little consideration of soil management guidelines for *V. vinifera* production (Havlin et al., 2012). Soil pH has been a commonly debated issue throughout the world with *V. vinifera* (Himelrick, 1991; Vasconcelos, 2001; Taylor et al., 2001; Kering and Kaps, 2011; Wooldridge et al, 2010). Most of the previous work has been done in solution culture; however, this is different from growing grape vines in soil since solution cultures are not buffered (Wright et al. 1989; Conyers et al., 1991). There is a need to characterize grape cultivar and rootstock tolerance to acid soil conditions for specific soil types and regions in NC. While management of soil acidity constraints in V. *vinifera* production by use of Al tolerance is one approach in NC, optimizing soil pH is critical for nutrient and water uptake, and overall health of the grape vine. The goal of this study is to establish a protocol for evaluating soil acidity impacts on *V. vinifera*.

Materials and Methods

A greenhouse experiment was conducted over a six-month period, March to August, in 2011 and 2012. A Norfolk sandy loam soil (Fine-loamy, kaolinitic, thermic Typic Kandiudult), soil pH of 4 and screened to particle size of 2-mm was obtained from the North Carolina Department of Agriculture and Consumer Services (NCDA&CS). It was originally collected at Central Crops Research Station in Clayton from a stand of loblolly pines. The soil was remixed for 15 minutes to insure uniformity using a Twister Batch substrate mixer. The homogenous soil was split into twenty-7.5 kg portions. The 20 portions were divided randomly into five replications of four pH levels (4, 5, 6, and 7). Each portion of soil was represented by one of twenty, 61-cm long, 10-cm diameter, clear plastic tubes obtained from Visipak Corporation; tubes were replaced yearly. Soil pH adjustment was different between the two years. Soil pH and fertility were increased using all reagent grade materials.

In 2011, soil pH adjustment (lime requirement) was estimated using a modified soil titration method with 40 g soil and 40 g DI water (Sims, 1996). Soil acidity was estimated by pH titration with 0.096N NaOH. Normality of the NaOH was obtained by acid-base titration, using acetic acid ($HC_2H_3O_2$) and phenolphthalein (Khopkar, 1998). Soil was stirred manually for one minute and left to equilibrate for 15 minutes between titrations. Lime rates consisted of 0.4 (pH 5), 1.1 (pH 6), and 2.2 g $CaCO_3$ $tube^{-1}$ (pH 7) in 2011. Desired pH levels were not attained by this method.

In 2012, soil pH adjustment was accomplished by using a soil-lime incubation method (Sims, 1996). Soil from 2011 was used in the soil-lime incubation study. Using 100 grams of soil in 200 cm^3 vials on each target pH level (soil pH 5, 6, and 7), $CaCO_3$ (0, 32, and 64 mg $CaCO_3$ for pH 5; 0, 48, and 96 mg $CaCO_3$ for pH 6; and 0, 71, 142 mg $CaCO_3$ for pH 7) was mixed with 22 mL of DI water by hand using a glass lab stir rod. Three replications were done. After a one-week incubation in the lab, samples were dried at 70°C and pH was determined in a 1:1 (v:v) soil to DI water. A linear relationship was established between the quantity of $CaCO_3$ added and soil pH to determine lime needs. Rates were as follows: 3.9 g $CaCO_3$ $tube^{-1}$ (pH 5), 7.7 g $CaCO_3$ $tube^{-1}$ (pH 6), and 11.7 g $CaCO_3$ $tube^{-1}$ (pH 7).

Most fertilizer was applied with the $CaCO_3$. Soils were mixed for 20 minutes in a two-shell blender to insure even distribution of fertilizer and $CaCO_3$. In 2011, fertilizer was added prior to planting. Approximately 71 mg N, 117 mg P, and 291 mg K $tube^{-1}$ with P and K applied according to NCDA&CS soil test recommendations. In 2012, approximately 164 mg N, 364 mg P, 597 mg K, 48 mg Mg, and 64 mg S $tube^{-1}$ were applied. Fertilizer rates were adjusted in 2012 due to severe nutrient deficiency symptoms that developed in 2011. In 2012, additional macro and micro-nutrients were added as nutrient solution drenches after planting due to Fe and/or Mn deficient symptoms and concerns of other nutrient inadequacy. Two nutrient solutions were used. On June 9th and June 19th 100 mL of nutrient solution per tube to provided approximately 27mg N, 60 mg P, 80 mg K, 50 mg Mg, 36 mg Zn, 18 mg Mn, 18 mg Cu, 44 mg Fe, and 7 mg B $tube^{-1}$. On July 25th and August 5th, a nutrient solution was applied in the same manner. The second nutrient drench consisted of 100 mL $tube^{-1}$, and provided approximately 80 mg N, 40 mg P, 60 mg K, 20 mg Mg, 6 mg Mn, and 11 mg Fe $tube^{-1}$.

The 20 tubes (four pH levels, five replications) were arranged in a completely randomized design. Approximately 75% of the tubes volume were filled with the selected soil treatment two weeks before transplanting *V. vinifera* into designated treatments. Distilled water (650 mL) was added to each tube to react the $CaCO_3$ with the soil before transplanting.

Prior to planting in the treated soil, 25 vines of *V. vinifera* ‘Cabernet Sauvignon’ grafted on 101-14 root stock (Sunridge Nursery) were planted in Fafard 4P potting media for two weeks to encourage bud break and growth. The most uniform vines were removed from the potting media; roots were washed with deionized (DI) water; and vine roots were carefully positioned in tubes using the amended soil. A 5 to 6-cm head space was left at the top of the tube for watering.

Plants were irrigated by adding DI water according to soil weight. *V. vinifera* were watered every two to three days to bring soil water to field capacity; water usage depended on sun exposer, temperature, and plant growth. Water rates were determined by obtaining a water retention curve using pressures 50; 100; 330; 500; 1,000; 5,000; and 15,000 cm. Field capacity was assumed to be 100 cm for sandy soil, while permanent wilting point was assumed to be at 15,000 cm (Lambers et al., 2008). To avoid excess water and potential

nutrient loss, water content did not exceed 0.19 cm^3 cm^{-3}. The maximum weight to obtain field capacity for each tube was 8.15 kg.

Harvest of vines occurred on August 11, 2011 and August 25, 2012. Vine shoot height was measured from the soil surface to the tallest branch. Tubes were cut to allow plant roots to be separated from the soil. Roots were washed with DI water and fresh weight was obtained after air drying. Plants were divided into leaf, stem, and root tissues. All tissue samples were dried at 75°C for 24 hours and then dry weight was obtained. A Mini Wiley Mill (Thomas Scientific) was used for grinding leaf tissue to 1-mm diameter and a model 4 Wiley Mill was used to grind woody tissue (roots and stems) to 2-mm diameter. Samples were then analyzed by Agronomic Division-NCDA&CS for nutrient content. Total N concentration was determined by oxygen combustion gas chromatography with an elemental analyzer (NA1500; CE Elantech Instruments; Milan, Italy) (Campbell, 1992). Total P, K, Ca, Mg, S, B, Cu, Fe, Mn, Al, Zn, and Na concentrations were determined with an inductively coupled plasma (ICP) spectrometer (Donohue & Aho 1992; USEPA 2001) (Optima 3300 DV ICP emission spectrometer; Perkin Elmer Corporation; Wellesley, MA) after open-vessel HNO_3 digestion in a microwave digestion system (CEM Corp.; Matthews, NC) (Campbell & Plank, 1992).

The soil was air-dried in the greenhouse for seven days then homogenized in a two-shell blender before taking subsamples. Subsamples were taken to Agronomic Division-NCDA&CS and dried at 54°C for 24 hours. Mehlich-3 extractable P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, and Al were analyzed with ICP-AES (Mehlich, 1984). Soil pH was determined on a 1:1 soil/water volume ratio. Cation exchange capacity (CEC) was determined by summation of base cations (excluding sodium) and buffer acidity as measured by Mehlich buffer (Mehlich, 1976). Base saturation was determined as the percentage of CEC occupied by basic cations (excluding Na).

JMP Pro 10 (SAS software) was used for statistical analysis of all data. Analysis of variance (ANOVA) was used to obtain significance of treatment effect with mean separation using Tukey HSD. Data across years were not combined in the statistical analysis, due to the differences in soil pH treatments between years.

Results and Discussion

Initially, overall soil fertility and pH were low (Table 1-1). Soil pH was a 4.2 with a 32% base saturation of before any lime treatments were established. Soil Mehlich 3 P and K were consider to be very low before addition of fertilizer. Established soil pH levels were different between the two years (Figure 1-1). Soil pH at the end of 2011 study ranged from 4.0 to 4.9 and did not achieve the target pH values. In 2012, target soil pH was achieved and ranged from 4.0 to 6.9. In 2011 the titration method provided inaccurate lime rates. In 2012, the titration versus the soil-lime incubation methods were compared (Figure 1-2). The soil-lime incubation method achieved the desired target soil pH of 7, while the highest pH obtained with the titration method was 5.2, even with the addition of a longer equilibration time. The soil-lime incubation method resulted in very accurate establishment of target soil pH (Figure 1-3); it is important to note that the initial pH without lime addition in Figure 1-3 was the upper end of pH achieved with lime treatment 2011 for respective target pH. Soil nutrient additions were increased in 2012 due to the severity of nutrient deficient symptoms observed in 2011 and at the beginning of 2012. Deficiency symptoms in 2011 were likely due to excessively low pH, in addition to limited ability of the low CEC sandy soil to supply nutrients and low soil volume per plant. With the increase in fertility, nutrient deficiency was less noteworthy in 2012 (Figure 1-4). Since pH targets were not achieved in 2011, plant growth and nutrient data, along with stem data from 2012 did not have noteworthy results. These data are not discussed and are found in the appendix (Appendix 1-1, 1-2, 1-3, 1-4, 1-5, and 1-6)

Plant Growth

Total biomass was lowest at target pH 4 as expected (Table 1-2), with growth being severely limited by soil acidity. No significant differences in biomass occurred at the higher target pH levels 5, 6, and 7. Looking at the individual tissues; leaf, stem, and root biomass were generally increased the most as target soil pH increased from 4 to 5, although significant biomass differences in each tissue type above target pH 4 did not always occur (Figure 1-5).Vine height varied considerably among target pH levels with no direct treatment effect seen. For unknown reasons, height at target soil pH 6 was not significantly different

than at pH 4. Results in 2012 may have been influenced by powdery mildew disease pressure in addition to nutrient availability as related to target soil pH.

Tissue Al & Macronutrients

Leaf and root Al concentrations decreased significantly with increasing soil pH only up to the target pH of 5, above which there were no further statistical differences (Table 1-3 & 1-4). An increase in pH by the first lime addition decreases Al in the leaf and root by 80% and 50% respectively. Toxic leaf Al concentration (255 mg kg^{-1}) was likely observed at soil pH 4. Leaf Al concentrations as low as 30 mg kg^{-1} can be defined as toxic in sensitive plants (Rout et al., 2001).

Leaf N varied considerably among target pH levels with the highest N concentration at target soil pH 6 and the lowest at 5 (Tables 1-3). There was significant difference observed between target soil pH 5 and 6. Root N concentrations significantly decreased as target soil pH increased from 4 to 5 (Tables 1-4). There was no significant increase in N above target soil pH 5.

There were significant differences in leaf and root P concentrations; however, the difference in P varied considerably among target soil pH levels (Tables 1-3 & 1-4). Highest P leaf concentration was observed at target soil pH 6 followed by soil pH 4. Target soil pH 4 and 6 in *V. vinifera* had the least biomass among the pH treatments, this potential could result in dilution of P as growth increased in the other treatments. Root P concentrations were greatest at soil pH 4 and lowest at soil pH 5. Symptoms of P deficiency were observed to be more severe in 2011versus 2012, and symptoms were noted to increase with decreasing soil pH (Figure 1-6). Increases in P fertilizer helped reduce symptoms in 2012.

Leaf Mg concentrations decreased significantly as soil pH increased. The lowest concentration was found at target pH 7 and the highest level at target pH 4, with no significant difference between target pH 5 and 6. Leaf K concentration was lowest at target pH 4 with similar K concentrations at higher target pH above 4 (Tables 1-3). As opposed to leaf tissue K concentration, root K concentration at target soil pH 4 was significantly lower in comparison to the other target soil pH levels (Table 1-4). Visual K deficiency symptoms were greatest in 2011 compared to 2012 (Figure 1-6). Target soil pH levels had no effect on root Mg concentration. Leaf and root Ca concentrations increased significantly with

increasing soil pH; no difference in leaf Ca concentration occurred beyond target pH 6. The additional $CaCO_3$ at higher Target soil pH levels resulted in greater available Ca and consequently increase in leaf and root Ca concentration. The increase in Ca likely antagonized uptake of K and Mg, even though additional K and Mg were supplied (Figure 1-7 & Figure 1-8).

There was a significant decrease in leaf tissue S concentrations between target pH 4 and higher target levels (Table 1-3). The decrease in leaf S occurred even with the addition of $MgSO_4$ in 2012. There was no response in root S concentration with increasing soil pH (Table 1-4).

Tissue Micronutrients

As expected, there was a significant reduction in leaf Fe, Mn, and Zn concentrations as soil pH increased, due to lower availability at higher pH (Table 1-3). Both Mn and Zn concentrations could be considered excessively high in the leaf tissue; Mn and Zn concentrations of 300 mg L^{-1} in leaf tissue can be considered excessive or toxic in most plants (Vitosh et al., 1994; Reichman, 2002). Mn leaf concentrations were well over 950 mg kg^{-1} at target soil pH 4, while Zn leaf concentrations approached 550 mg kg^{-1} at target soil pH 4. Manganese and Zn toxicities were suspected at these levels. Excessive Mn and Zn levels may have suppressed other micronutrient uptake such as Cu and Fe. Lime had no effect on leaf Cu concentration. There were also significant decreases in root Mn and Zn concentrations with increasing soil pH, possibly due to better metabolism and translocation (Table 1-4). The varying root Fe concentrations were likely not related to treatment but potential soil contamination of root tissue. Root Cu levels varied but without plausible reason.

Leaf B concentrations decreased significantly with increasing soil pH (Table 1-3), while root B concentrations significantly increased with increasing soil pH (Tables 1-4). Increasing root B concentration was opposite of what would be expected as soil pH increased. Normal solubility of B decreases with increasing soil pH. Visible symptoms which were suspected to be B toxicity were noticed in 2012 after the second nutrient solution drench containing B. Symptoms included abnormal cupping of younger leaves, smaller leave size, shortening of the internodes, and termination of terminal leader (Figure 1-9). These

symptoms decreased with increasing soil pH. For a number of crops, leaf tissue containing 250 mg kg^{-1} B is approaching toxic levels (Nable et al., 1997), where B leaf levels in 2012 ranged from 245 to 525 mg kg^{-1} B.

Soil Al & Macronutrients

Mehlich 3 Al concentration decreased significantly as soil pH levels increased (Table 1-5). The highest concentration was observed at target soil pH 4 and the lowest Al concentration was observed at target soil pH 7, with a decrease of approximately 347 mg L^{-1} Al (37%) as soil pH increased from 4 to 7.

Mehlich 3 P, Mg, and S concentrations were lowest at target soil pH 7 (Table 1-5). The highest level of K was found at target pH 4 with variability in K at higher target pH levels. The lowest P concentration was found at target pH 7 with no significant difference below target pH 6. Note the highest P concentration was observed at soil pH 4, while the lowest P concentration was observed at target soil pH 7. A plausible explanation for these nutrients being lowest at highest pH (7) is due to the greater biomass and nutrient uptake at this pH. The decrease in K and Mg concentrations were also mentioned in the plant tissue section; decrease in plant tissue K and Mg were more related to increase in soil pH and possible interference in plant uptake.

Mehlich 3 Ca concentration increased significantly with increasing soil pH as related to use of $CaCO_3$ as the lime source (Table 1-5). No $CaCO_3$ or other source of Ca was added to target soil pH 4. Higher soil Ca corresponded to increased tissue Ca concentration. This increase in soil Ca likely reduced K and Mg uptake in the plant. As Ca increased, the ratio of Ca:K and Ca:Mg increased in the soil which probably lead to the antagonism of K and Mg uptake in the leaf tissue (Figures 1-10 & 1-11).

Soil Micronutrients

Mehlich 3 Fe, Mn, Zn, and Cu concentrations decreased significantly with increasing soil pH (Table 1-5). High levels of micronutrients seen in 2012 were in response to the addition of micronutrients applied as a liquid fertilizer at watering. Even with the addition of Fe in 2012, target soil pH 7 greatly reduced Fe availability in comparison to other soil pH treatments. Mn concentration was reduced by half as target soil pH increased from 4 to 7. This reduction in available Fe and Mn in the soil could have led to the deficiency symptoms

observed in 2012, with target pH 7 showing the most symptoms. High soil Mn and Zn in soil pH 4 may have led to toxic levels of Mn and Zn in the leaf tissue.

Conclusion

A target soil pH of 5 or greater resulted in improved plant performance in *V. vinifera* on 101-14 rootstock. Even in 2011, with an increase in target soil pH from 4 to 5, a distinguishable increase in plant performance was noted. Increasing target soil pH had an impact on decreasing Al concentration within all plant tissues. Increasing target soil pH to 5, dramatically dropped Al concentration in plant tissue and soil. A fourfold decrease in Al concentration was detected in the leaf tissue as target pH increased from 4 to 5. Target soil pH 5 or greater resulted in plant biomass and height being maximized. Target soil pH 7 or greater could significantly reduce available Fe, Mn, and Zn in the soil and plant tissue. Toxicity associated with Al, Mn, and Zn possibly influenced plant growth at target pH 4. Excessively high Mn could have suppressed Fe uptake which possibly lead to the Fe deficiency symptom seen in 2012. Increasing soil pH with $CaCO_3$ alone resulted in increased Ca saturation in the sandy soil, which adversely impacted plant uptake of several nutrients and resulted in a detectable antagonism with uptake of K and Mg. A balance between soil K:Ca:Mg appears to be very important in regulating the uptake of the three nutrients. In this experiment leaf tissue P concentration did not increase with increasing soil pH; however, P is highly regulated by soil pH, forms compounds with other ions, and becomes less soluble in alkaline or acid soils. There was evident interveinal reddish purpling in the lower leaves, located mostly on the outer edge with an inward movement as symptom got worse. These symptoms resembled P and K deficiency symptoms and increased with decreasing soil pH. However, since leaf P concentration decreased with increased biomass, the symptoms are more complex to explain than just because of lower pH and soil P. In 2012, toxicity associated with B appeared to be greatly influenced by soil pH, with target soil pH 4 having doubled the concentration of B in the leaf tissue in comparison to 7. The B concentrations in 2012 had a negative impact on plant growth. Overall the study showed positivity result with increasing soil pH.

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Table 1-1. Starting soil fertility before setting pH treatments.

	CEC	BS	W/V	P	K	Ca	Mg	Mn	Zn	Cu
Soil pH	meq 100 cm^{-3}	%	g cm^{-1}	----------mg L^{-1}----------						
4.2	4.4	32	1.6	26.4	37.1	167.2	53.5	3.2	2.1	0.72

Table 1-2. Effects of soil pH on *V. vinifera* height and total biomass in 2012.

Target Soil pH	Height[1]	Biomass[1]
	---------*cm*---------	--------*g*--------
4	73.7b	45.2b
5	111.4a	74.6a
6	83.3b	68.8a
7	100.5ab	82.8a
p	0.0056*	0.0015*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table 1-3. Effects of soil pH on *V. vinifera* leaf tissue nutrient concentrations in 2012.

Target Soil pH	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
	---------- % ----------					
4	3.02ab	0.19ab	2.26a	0.55c	0.41a	0.80a
5	2.30c	0.16b	1.60b	1.08b	0.34b	0.39b
6	3.20a	0.20a	1.68b	1.50a	0.29b	0.35b
7	2.62bc	0.17ab	1.43b	1.68a	0.23c	0.29b
p	0.0013*	0.0194*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	Al[1]
	---------- *mg kg^{-1}* ----------					
4	480ab	979a	546a	9.9a	525a	255.0a
5	588a	761ab	293b	8.6a	342b	51.8b
6	397bc	519bc	166b	11.3a	316bc	43.7b
7	280c	360c	160b	9.6a	245c	22.9b
p	< 0.0001*	0.0011*	< 0.0001*	0.1464	< 0.0001*	< 0.0001*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table 1-4. Effects of soil pH on *V. vinifera* root tissue nutrient concentrations in 2012.

Target Soil pH	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
	%					
4	2.04a	0.14a	0.51b	0.26d	0.12a	0.35a
5	1.42b	0.11b	0.84a	0.59c	0.13a	0.32a
6	1.41b	0.12ab	0.82a	0.95b	0.13a	0.32a
7	1.41b	0.13ab	0.77a	1.18a	0.12a	0.29a
p	< 0.0007*	0.0051*	0.0034*	< 0.0001*	0.6368	0.3963
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	Al[1]
	mg kg^{-1}					
4	1694a	266.2a	559a	105.1a	30.1b	2360a
5	1135b	160.9b	380b	59.1b	26.1b	1153b
6	1473ab	156.8b	291bc	87.4ab	37.0ab	839b
7	1688a	80.0c	175c	86.8ab	49.5a	736b
p	0.0192*	< 0.0001*	< 0.0001*	0.0113*	0.0036*	< 0.0001*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table 1-5. Mehlich 3 soil test results of soil pH treatments at end of study in 2012.

Target	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
Soil pH	---------- *mg L^{-1}* ----------				
4	131.8a	189.8a	84.2d	56.6a	135.8a
5	128.0a	95.0bc	438.6c	48.4a	135.6a
6	122.6a	111.4b	873.2b	50.2a	143.8a
7	91.6b	78.8c	1048.0a	37.4b	99.8b
p	< 0.0001*	< 0.0001*	< 0.0001*	0.0002*	0.0004*
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	Al[1]
	---------- *mg L^{-1}* ----------				
4	269a	10.30a	12.82a	5.78a	932a
5	249ab	8.02b	11.54ab	5.58ab	880a
6	226b	7.36b	10.64b	5.14b	782b
7	176c	5.32c	7.24c	4.12c	585c
p	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

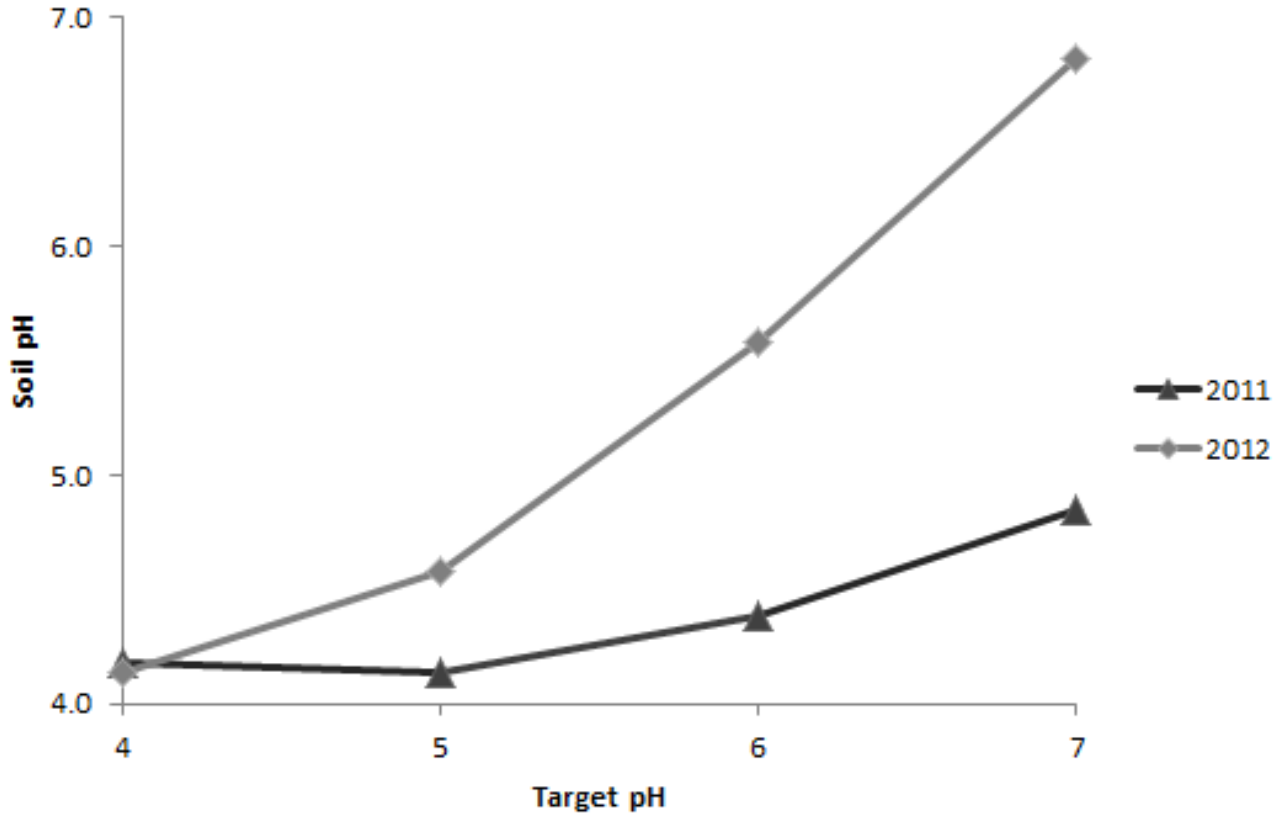

Figure 1-1. Soil pH after harvest in comparison to target soil pH.

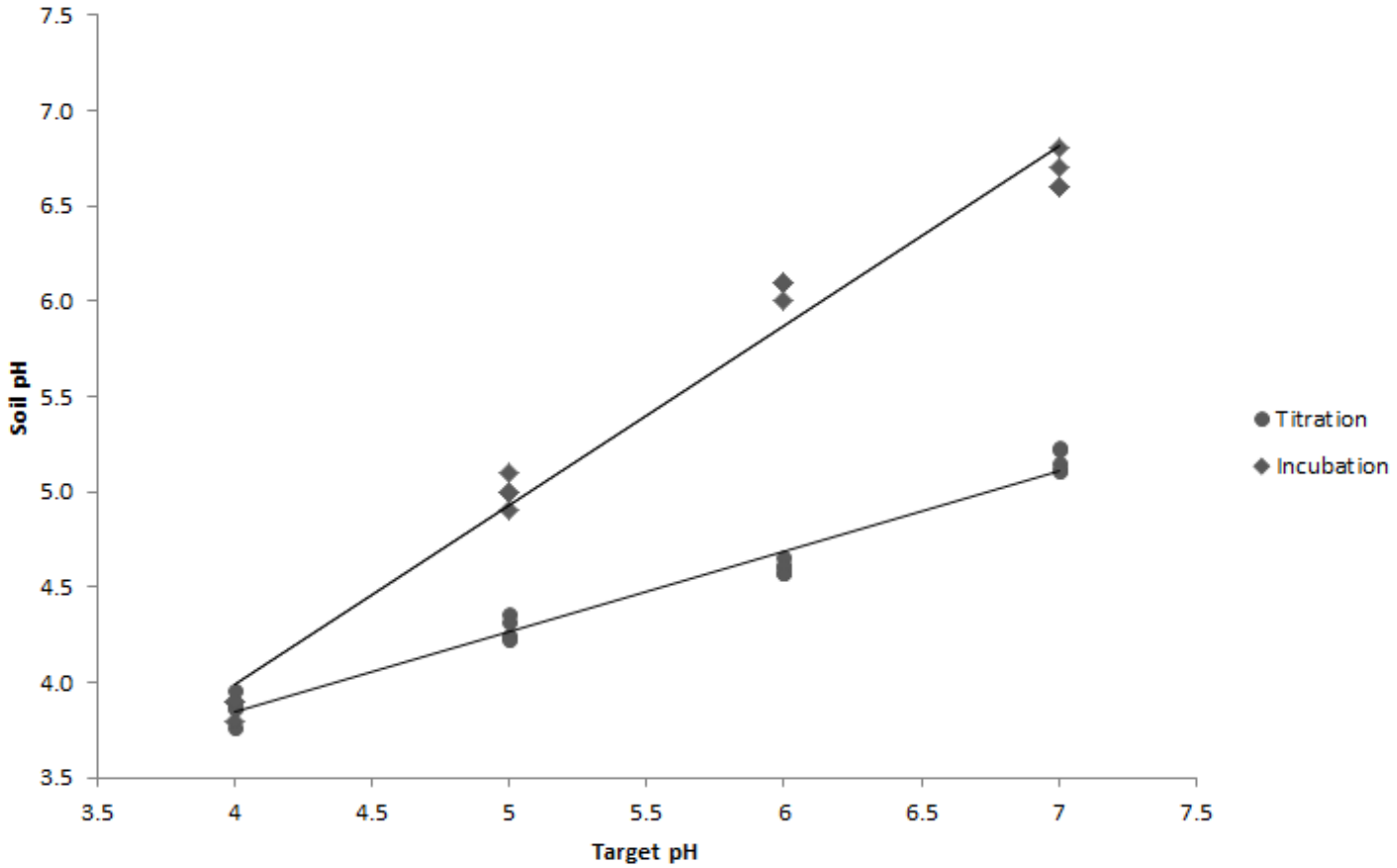

Figure 1-2. Comparison of titration versus soil incubation in achieving target soil pH.

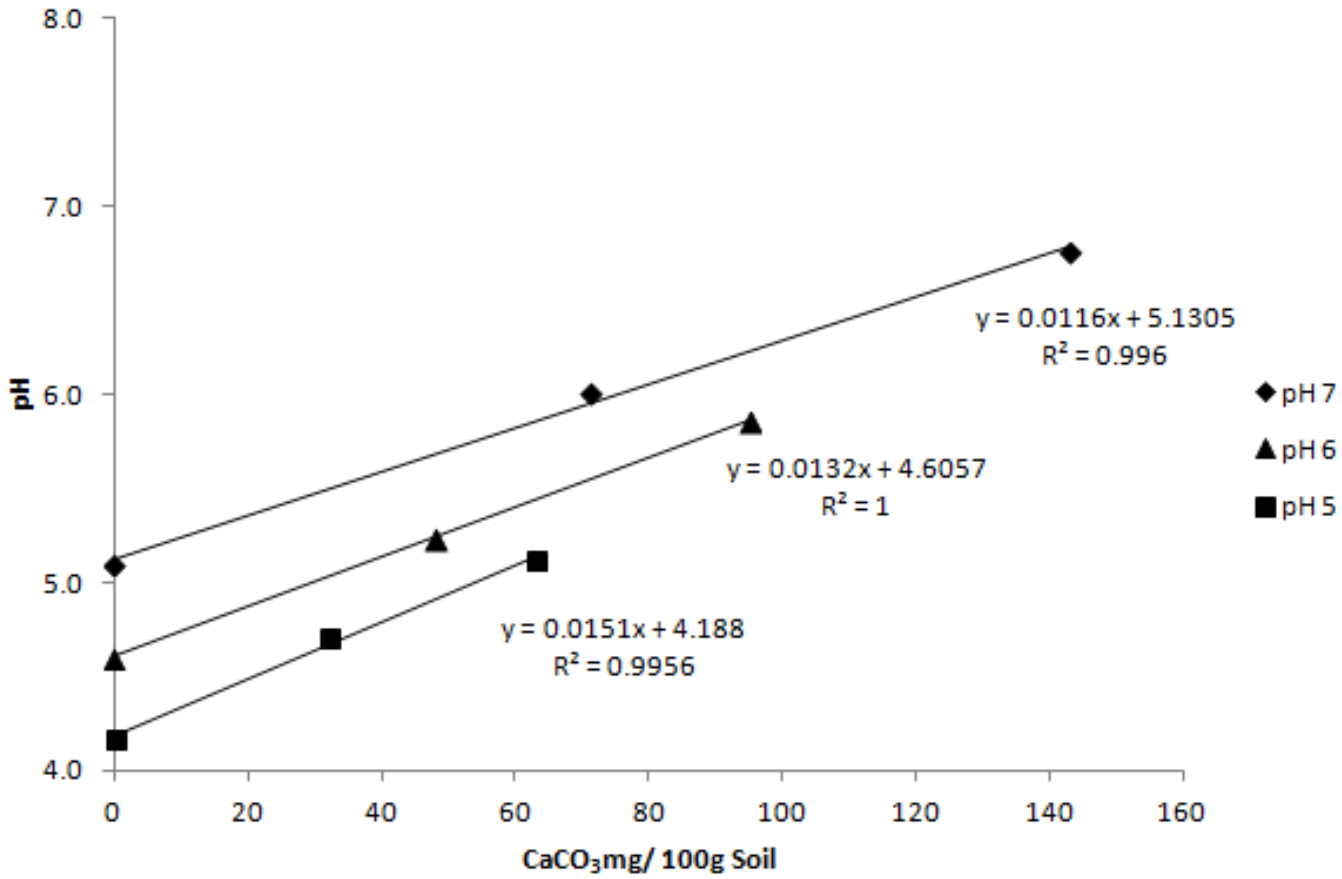

Figure 1-3. Soil pH response to $CaCO_3$ addition in 2012 soil incubation study.

Figure 1-4. Comparison photos of *V. vinifera* plant growth between 2011 (top) and 2012 (bottom) at the target pH levels- 4, 5, 6, and 7.

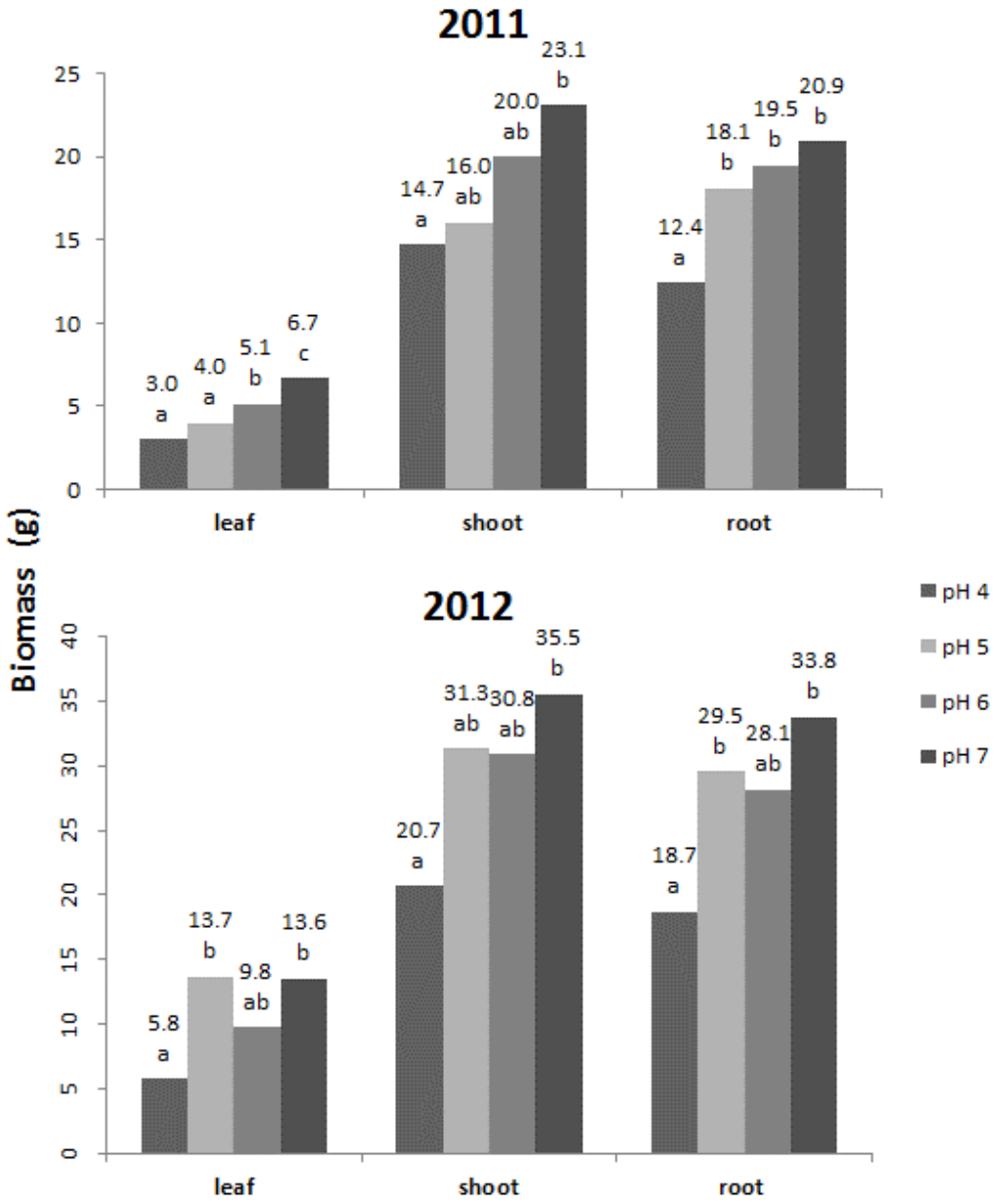

Figure 1-5. Soil treatment pH effects on the leaf, stem, and root tissue biomass in *V. vinifera*. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

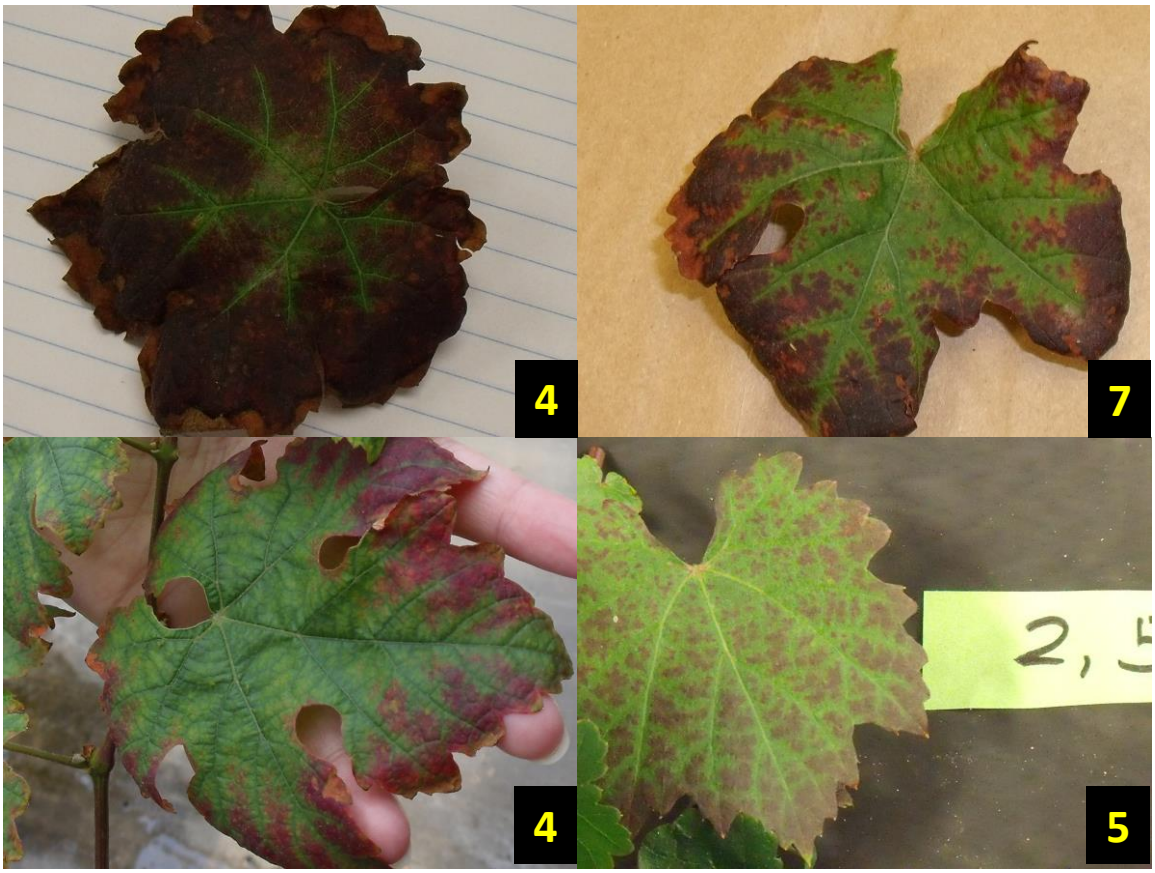

Figure 1-6. Nutrient deficiency symptoms and how they compare between 2011(top) versus 2012 (bottom) at target pH of 4, 5, and 7.

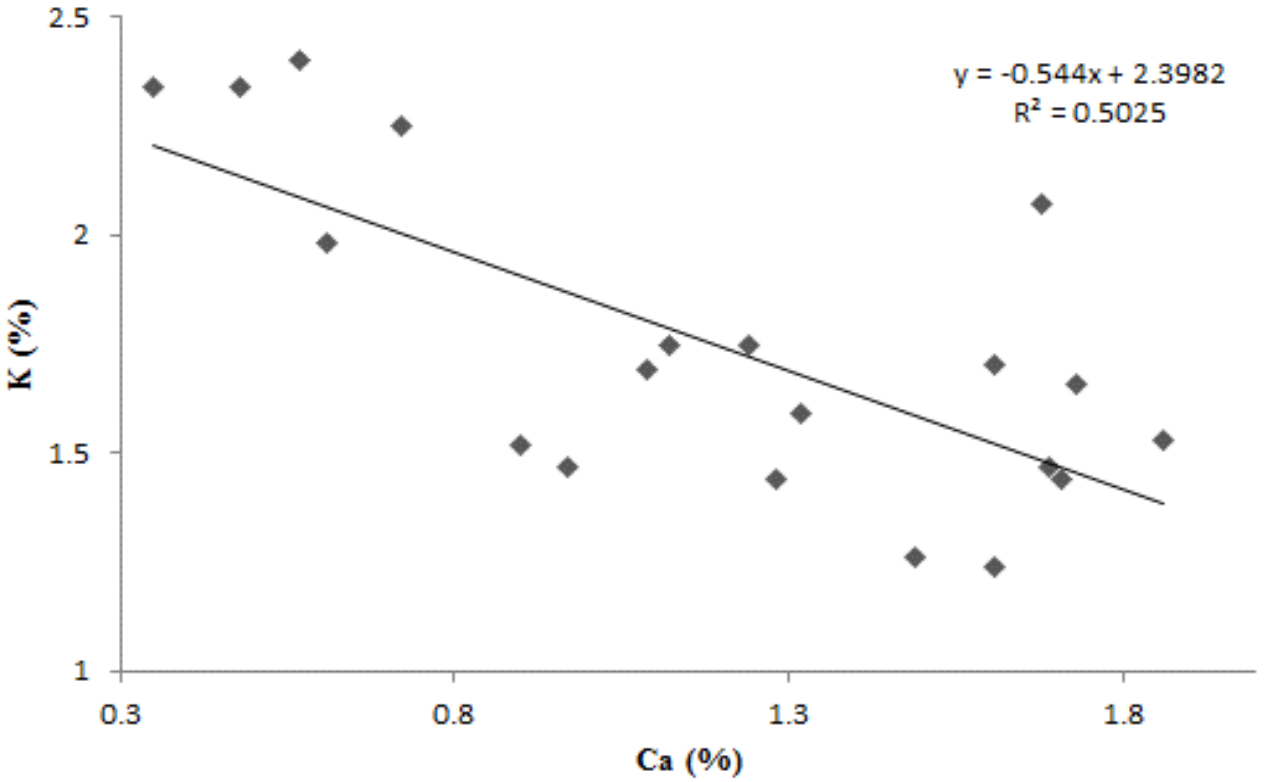

Figure 1-7. Influence of increasing Ca concentration on K concentration in the leaf tissue (2012).

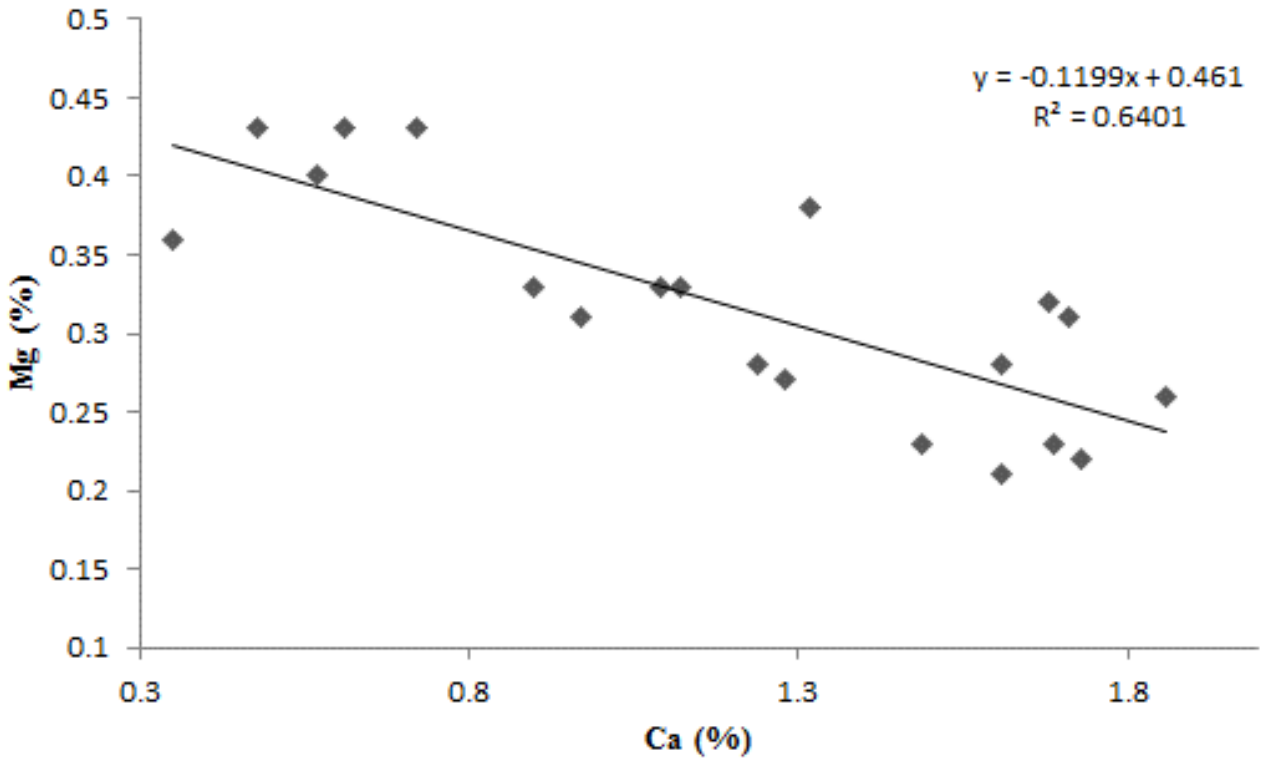

Figure 1-8. Influence of increasing Ca concentration in the leaf tissue on Mg concentration (2012).

Figure 1-9. A noticeable B toxicity symptom was noted in all treatments (target pH 5 above) in 2012.

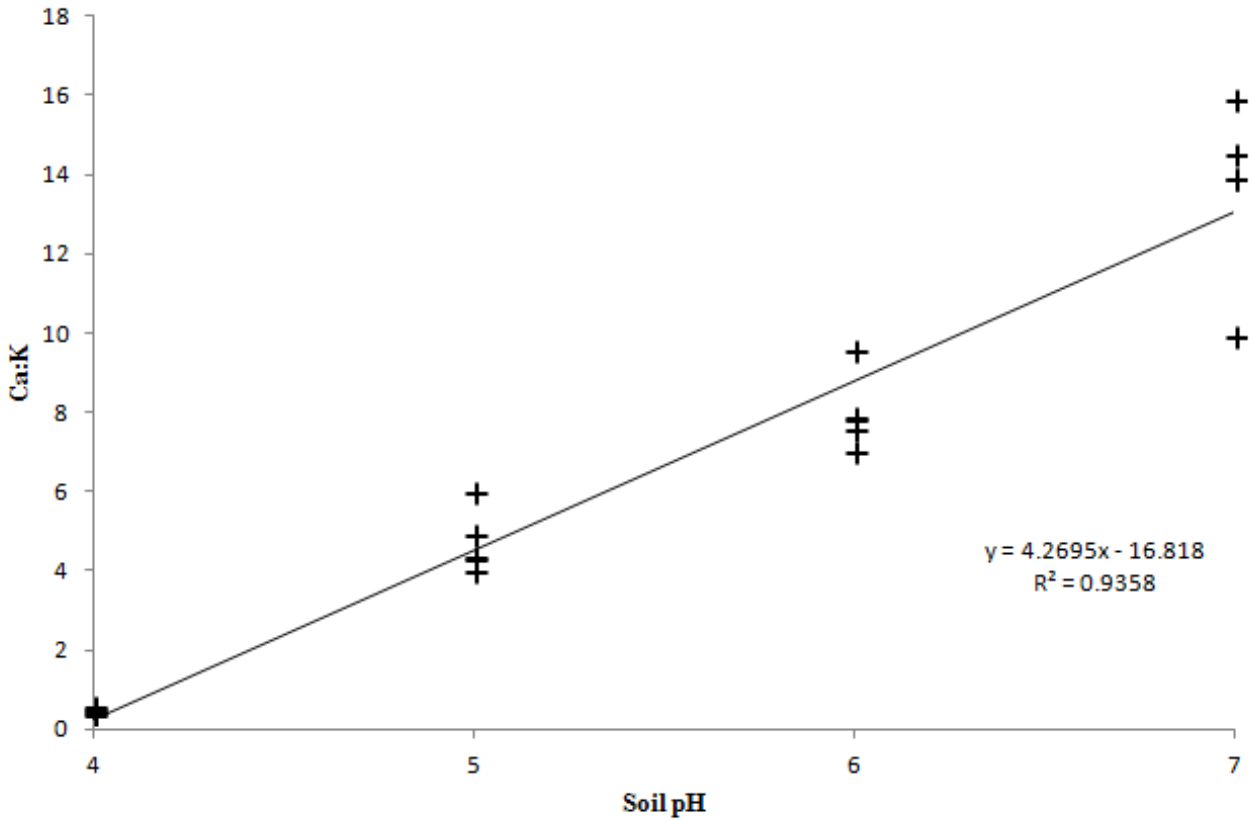

Figure 1-10. Influence of increasing soil pH and its effects on soil exchangeable Ca:K ratio.

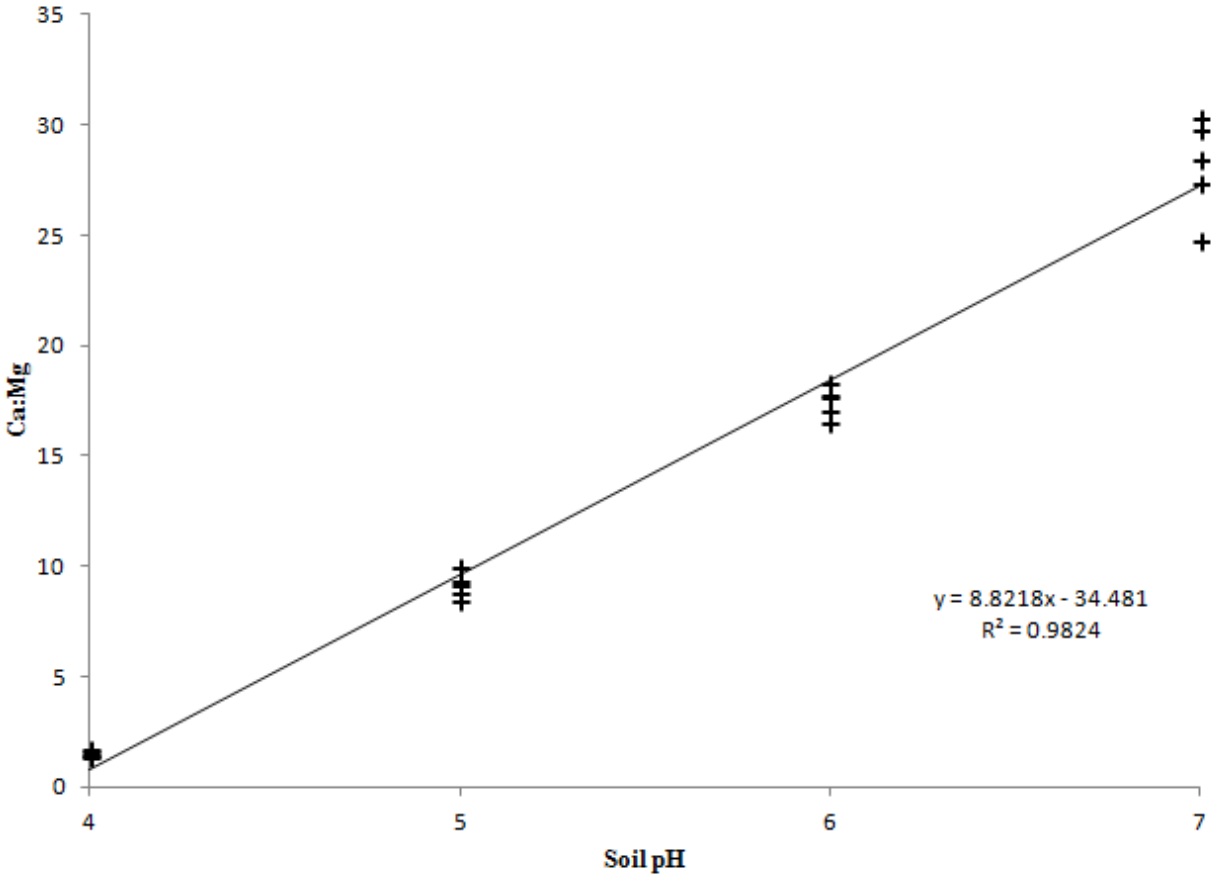

Figure 1-11. Influence of increasing soil pH and its effects on soil exchangeable Ca:Mg ratio.

Chapter 2

Effects of nitrogen loading on plant growth and nitrogen concentration in container grown trees

Abstract

Nitrogen (N) is one of the most important nutrients needed among plant species for growth and development. While tree species vary in their N requirement, few N management guidelines are available for trees in potted soilless media culture. The objective of this study was to evaluate three commonly grown container tree species and their ability to utilize (store) N. *Tilia cordata, Acer rubrum,* and *Cedrus deodara* were grown in pine bark nursery media at 3, 9, 18, and 27 g N pot^{-1} of slow-release N. Differences in the use of slow-release N varied with tree species. *Cedrus deodara* was a low N user, with little or no difference in growth or tissue N concentration. *Tilia cordata* and *Acer rubrum* both were considered high N users. With *Tilia cordata,* increasing N rate increased stem and root N concentration and N use efficiency. *Acer rubrum* root N concentration and growth increased with N rate. Our conclusion is that each of these three tree species should be fertilized with sufficient N in the nursery environment to maintain vigor and adaptability during the stress of transplanting into a landscape or forestry site.

Introduction

Production of newly planted container trees in nurseries or landscape settings can often be limited by low nitrogen (N) levels throughout the establishment period associated with transplanting. This is due to poor N uptake in the newly planted trees, low soil N levels, and varying needs among species for N that may not be met in a nursery culture system (Werken, 1981; Timmer, 1996; Watson, 2005; Salifu et al., 2009; Barker, 2010; Schulz et al., 2011). Trees grown in the nursery and planted into the landscape frequently require a lengthy establishment period, in which N may not be readily taken up by the plant. Factors affecting establishment include tree size, root-ball condition, and backfill material (Werken, 1981;

Watson, 2005; Day and Harris, 2007). Trees that are deprived of N exhibit poor growth and reduced survivability.

Nutrients needed for plant growth can be evaluated using four different diagnostic categories: deficiency, critical range, luxury consumption (sufficiency), and toxicity. In container trees, N concentration for each of these categories varies with plant species, age, fertilizer type, and media type (Dreistadt, 2001). Sufficiency ranges of container grown trees are not well documented and most ranges have been adapted from established mature trees in soil (Perry & Hickman, 2001). With luxury consumption of N, also called N-loading, in which plants take up N in amounts greater than plant requirement, the N can be stored for later use (Millard, 1987; Barker and Pilbeam, 2007). One technique used to overcome low N uptake in the establishment period is to apply N to encourage N-loading. This method of supplying N in excess of demand for current growth before transplanting induces luxury consumption that increases tissue N levels without significant changes to biomass yield (Mullin and Bowdery, 1977; Timmer and Munson, 1991). Not all trees, however, are suited for N-loading. Larimer and Struve (2003) induced N-loading conditions with two ornamental tree species, and only one exhibited N-loading.

The distribution of N within the plant can vary with tree species. Normally, fine roots and leaf tissue store most of the N (85%), while wood tissue (99% of total plant weight) contains only 15% of the N (Coder, 1997). In trees, approximately 60% of tissue N is annually remobilized (Coder, 1997). Therefore, N reserves are very mobile and easy to store and remobilize for use when needed, for example, in the stressful establishment period. These N reserves are mainly in the form of nitrate (NO_3^-) or proteins. While high levels of ammonium (NH_4^+) within the tissue can cause toxicity, NH_4^+ is easily converted to NO_3^- in soilless media (Millard, 1987; Timmer and Munson, 1991, Timmer, 1996; Malik and Timmer, 1996; Xu and Timmer, 1999; Cabrera and Devereaux, 1999; Davis et al., 2000; Salifu et al., 2009; Barker, 2010).

Two main types of N fertilizer in the nursery industry are granular or liquid (fertigation), where granular N sources are the most common and can be incorporated pre-plant or topdressed after planting (Zinati, 2005). The majority of past research focused on fertigation to optimize N, whereas few studies have used granular slow-release fertilizers.

Slow-release N (SRN) is one of the most common fertilizer sources used in the nursery industry as growers use a single application to supply N throughout the growing season for optimum plant growth. Most SRN is encapsulated urea, either osmocote or polycote blends that release N through a membrane that may or may not itself be soluble (Trenkel, 1997). The use of SRN results in high recovery of the N fertilizer which reduces detrimental effects to the environment (Trenkel, 1997).

With the use of soilless media, standard industry N recommendations are based on container size with no adjustment for N requirements of individual plant species. For example, the *NC Agricultural Chemical Manual* indicates that the N requirement for container plants needs to be based on plant type and location, but gives no recommendations for specific species (Bilderback et al., 2010). Recommended application rates on a fertilizer label can range from 2 to 4 g N 3.78 L^{-1} pot, but this varies with fertilizer companies and product types. A normal slow-release N rate used by the industry is 3 g N per 3.78 L^{-1} pot in a growing season (Bilderback and Kraus, NCSU nursery specialist, personal communication, 2009). However, most agricultural crops have specific N rates for different plant species, yield levels, and soil types. In the *NC Agricultural Chemical Manual*, N rates for agriculture crops are based on a Realistic Yield Expectation (RYE) using an N factor (amount of N for crop specific yield) and soil type. In contrast, nursery crops have no yield goal, but have only aesthetic value that increases with size, mass, and rarity of the tree or plant (Rosenow and Fazio, 1993), no specific N rates are provided. Also, demand for a particular nutrient can change with time since it is influenced by changes in environmental factors that affect plant growth (Greenwood, 1976). As a result, N needs will vary greatly between plants depending on multiple factors associated with plant N response.

Under normal N fertilization rates, increased plant dry matter dilutes nutrient concentration in the plant; which maintains nutrient concentration at a relatively constant level (Barker, 2010). Modification of the fertilizer program is required to establish N-loading conditions. Ingestad and Lund (1979; 1986) were two of the first to study the effects of exponential N fertilization on plant biomass and plant N levels. Exponential fertilization increases N amounts applied over time as plants develop. The previous work on N-loading has been done mainly in container grown trees for forestry production, with a focus on

exponential fertilization using fertigation to improve transplant survivability (Timmer and Munson, 1991; Miller and Timmer, 1994; Timmer, 1996; Malik and Timmer, 1996; Salifu and Timmer, 2003; Salifu et al, 2009; Barker, 2010). Similar N management has been successfully conducted with ornamental container production (Cabrera and Devereaux, 1999; Stratton et al., 2001; Larimer and Struve, 2003; Cabrera, 2003; Conden et al., 2003; Evans et al, 2008). With optimum N-loading rates, nursery tree biomass production after transplanting was greater than other N management methods even though trees in some cases were the smallest at transplanting. Other situations with similar results have been documented where trees treated with higher N rates reached a point when growth rates leveled out but N concentration increased within the tissue compared to lower N rates. These N-loaded trees once, planted in poor fertility soils, performed better than their counterparts, even with the addition of N after planting (Ingestad, 1977; Xu and Timmer, 1999; Cabrera and Devereaux, 1999; Cabrera, 2003; Salifu and Timmer, 2003). This improved performance can be attributed to the remobilization of N reserves (Xu and Timmer, 1999).

In *Picea mariana*, N-loading increased plant N content 78 to 150% more than unloaded seedlings prior to planting; once out-planted, the N-loaded trees produced greater biomass than non-loaded trees at all sites (Timmer and Munson, 1991; Salifu and Timmer, 2003). With Red Oak (*Quercus rubra*) under six different N rates, dry weight increased to the maximum N rate of 400 mg L^{-1}, but total tissue N concentration decreased slightly after 200 mg L^{-1}. On the other hand, Red Maple (*Acer rubrum*) increased in both dry weight and N concentration with the highest N rate (400 mg L^{-1}). N-loading was achieved in Red Maple (*Acer rubum*) but was not observed in Red Oak (*Quercus rubra*) due to the decrease in tissue N concentration (Larimer and Struve, 2003). Growth of *Ligustrum* increased with increased N rate where maximum growth occurred between 50 and 250 mg N L^{-1}. At higher N rates, growth rates decreased with increasing tissue N concentration. In contrast, *Lagerstroemia* trees that received the highest N rate were the smallest; however, out-planting these trees produced the highest biomass due to the buildup of N reserves (Cabrera and Devereaux, 1999; Stratton et al, 2001). In a similar study, *Euonymus fortunei* increased N leaf concentration with increased N rates using fertigation; *Euonymus* also had higher biomass and a lower root:shoot ratio (Evans et al., 2008). With container grown *Ternstroemia*

gymnanthera, root tissue N plateaued at higher N rates than shoot tissue N. Shoot biomass plateaued at higher N rates than root biomass (Conden et al., 2003), showing the difference in N accumulation between plant parts.

Although needed for plant growth, N can pose a risk to the environment if not managed properly. N loss from soil occurs by denitrification, volatilization, and leaching (Trenkel, 1997). Nitrate (NO_3^-) is highly soluble in water and is not strongly adsorbed to the anion exchange capacity (AEC) due to NO_3^- having a low negative charge in comparison to other anions, and is easily leached out of the soilless media or soil (Havlin et al., 2005). Once in the water supply, NO_3^- is a health risk when consumed by humans, causing two health problems: methaemoglobinaemia (blue-baby syndrome) in infants, and stomach cancer in adults (Addiscott, 1996).

Best management practice (BMP) guidelines suggest NO_3^- in pour-through ranges from 15 to 25 mg L^{-1} for granular SRN and from 50 to 100 mg L^{-1} for SRN with fertigation (Bilderback, 2001). Generally, BMP guidelines suggest a range of 100 to 150 mg N L^{-1} with not over 50 mg L^{-1} being NO_3^- (LeBude and Bilderback, 2009). Pine bark media, which is the main soilless media used in the nursery industry, has even greater potential for N loss, with little or no AEC and a cation exchange capacity (CEC) of 10 to 13 meq 100 cm^{-3} (Krewer and Ruter, 2009). N losses in pine bark media must always be considered because the potential for N loss increases with increasing N rate. In other words, the uptake efficiency decreases as N rate increases. For example, the efficiency in N uptake in azaleas decreased with increasing N rate, but total plant N increased (Ristvey et al., 2007; Struve, 1995). One method to improve recovery of applied N and reduce N loss is the use of SRN that is already used extensively in the nursery industry (Furuta, 1976; Hershey and Paul, 1982; Trenkel, 1997). BMP's identify the N rate appropriate for optimal plant growth and N-loading while minimizing N leaching loss.

The nursery industry in the US had a value of $6.6 billion with nearly 202,343 hectares in production (USDA, 2009). The North Carolina nursery and greenhouse industry had cash receipts total over $384 million in 2010 (NCDA&CS, 2012). An important goal of the industry is to maximize profits by using resources wisely. For example a sound N management strategy is essential in the production of any agriculture or horticulture crop.

Therefore, a major focus of the nursery industry should be on N recommendations that result in optimum plant health and yield at the lowest production cost while reducing risk to the environment.

In this experiment, our objective was to investigate three commonly grown ornamental tree species and their ability to maximize N in the plant tissue, in a pine bark media, with increasing rates of slow-release granular N fertilizer. We hope to illustrate the need for species-specific N rates within soilless media. Optimizing N levels can save costs by avoiding the application of pre-plant N fertilizer in the landscape or field (Timmer and Munson, 1991), and increased survivability of transplants will reduce the cost of replacing plants with warranties or replacement contracts.

Materials and Methods

The three trees species used were *Tilia cordata* (Littleleaf Linden no specific cultivar); *Acer rubrum* "Autumn Flame" (Red Maple); and *Cedrus deodara* "Patti Faye" (Deodar Cedar). *Tilia cordata* and *Acer rubrum* were obtained from Carlton Plant LLC in Oregon and *Cedrus deodara* was obtained from Rushing Nursery in Alabama. One hundred seedlings of each dormant species were planted individually into 11.4 L pots with 100% pine bark media in late March, and were placed within a plastic covered greenhouse. Any broken or small seedlings were discarded from the experiment. Five unplanted seedlings from each species were chosen randomly to evaluate tissue N concentration before treatment. Following potting, each seedling was randomly assigned one of four N rate treatments. Drip stick micro-irrigation was used while trees were in the greenhouse (March 19 to July 1, 2010) followed by overhead irrigation in the field (July 1 to October 13, 2010). Drip irrigation was set for 3 times a day (9:00am; 1:00pm; and 6:00pm) at 15 minutes intervals. Overhead irrigation set for three times of day, same times as above, but interval increased to 20 minutes. Trees were placed in three species oriented, randomized complete block design for the duration of the experiment (March 19 to October 13, 2010).

Nitrogen rates were 3, 9, 18, and 27 g N pot^{-1}. There were 25 reps for each N treatment. All N treatments were topdress applied to the media surface on April 13, 2010. Fertilizers used in the experiment consisted of Osmocote 13-13-13 and polycoated urea 42-0-

0; both were 6 to 8 month SRN fertilizers, with N release rate into the substrate controlled by osmotic potential. Osmocote 13-13-13 was used at 23 g pot^{-1} to get 1.3 g P pot^{-1} and 2.5 g K pot^{-1}, while providing 3 g N pot^{-1}. The polycoated urea was used to increase N rates among the other treatments. Micronutrient were added using Harrell's booster plus at 14 g pot^{-1} to obtain 1.4 g Mg pot^{-1}, 1.4 g S pot^{-1}, 1.7 g Fe pot^{-1}, 0.14 g Mn pot^{-1}, 0.14 g Zn pot^{-1}, and 0.14 g Cu pot^{-1} (Harrell's LLC). Harrell's booster was added along with the other fertilizer as a topdress. A total of 3.19 kg of dolomitic lime was added to 0.765 cubic meters of pine bark media and mixed for 15 minutes in a Twister substrate mixer. Each 0.765 cubic meter of media potted 60 to 65 pots. Because the fertilizer used supplied in relativity low rate of K and no B in the micronutrient fertilizer, both were added using a liquid fertilizer solutions to increase levels within the soilless media. Liquid potassium sulfate (0-0-25) was added twice using 236 mL solution containing 1,000 mg K L^{-1} and 6 mg B L^{-1} (boric acid) per 11.4 L pot. The K + B solution was applied on May 5, 2010; the second application containing only K was applied on June 2, 2010.

Tree height (cm) was measured from the substrate surface to the highest terminal branch. Diameter (mm) was measured using a digital metric caliper tool at 5 cm above substrate surface for *Acer rubrum* and 6 cm above substrate surface for *Tilia cordata* and *Cedrus deodara*. Three measurements were taken around the stem to determine mean diameter. Initial measurements were taken on March 19 and 20 at the beginning of the experiment, while trees were still dormant, followed by a final measurement on October 1. The two measurements were used to compare the growth differences for height and diameter during the treatment period.

The pour-through method was used to evaluate the media soluble inorganic N levels (NO_3^- and NH_4^+), pH, and electrical conductivity (EC) of the leachate (Bilderback, 2001). Samples were collected on April 2-9, May 21-28, and August 16-23, 2010, using 325 mL of water. Leachate was collected in April before application of any fertilizer. Leachate samples were stored in a freezer (-2°C) until analysis. Samples were analyzed for pH and EC using a Fisher pH/EC meter (Wright et al., 1986; Thermo Scientific pH Electrode Handbook, 2009). Leachate NO_3^- and NH_4^+ concentrations were determined using a Lachat 8000 continuous flow chromatograph (U.S. EPA, 2004).

Five trees within each species from each treatment were randomly harvested on October 2, 6, and 13, 2010. Tree roots were removed from the media and roots were washed using three, 18.9 L-buckets of deionized water. Plants were left to air dry for approximately one hour and then measured for fresh weight. After measuring fresh weight, trees were separated into leaves, shoots, and roots. Plant material was dried in an oven at 75°C for 24 hours and then weighed for dry weight. Leaf tissue was ground to 1-mm diameter in a Mini Wiley Mill; a model 4 Wiley Mill was used to grind woody (stem and root) tissue to a 2-mm diameter, for N analysis.

A modified Total Kjeldahi N (TKN) method was used to determine plant tissue N (Bremner, 1996; Persson, 2008). Modifications to this method were the use of 0.5 g of plant tissue, 30 mL of 95 % concentrated H_2SO_4, 8 g K_2SO_4, and 0.2 g $CuSO_4$ per tube. Note Cu replaced mercury (Hg) as the catalyst, because of safety concerns using Hg. One g salicylic acid per tube was used for the recovery of NO_3^- and nitrite (NO_2^-). Digestion was complete when the solution turned clear with a blue tint (blue tint materialized from the Cu). At the point of solution clearing, recovery of N can range from 95% to 100% (Persson, 2008). Digestion time ranged from 4 to 6 hours, with the temperature starting at 200° C and finishing at 385°C. Blank samples were used to evaluate contamination, while check samples were used to check the completion of the digestion and whether or not N was fully recovered.

Estimation of Nitrogen Use Efficiency (NUE) was used to evaluate uptake of N with increasing N rates, using the lowest rate of 3 g N pot^{-1} as a control to evaluate the other N rates. NUE was calculated by taking the average of total plant N (N_T) within increasing N rates subtracted by the average total plant N at 3 g N pot^{-1} (N_B) divided by N fertilizer difference between total N fertilizer added (N_{FT}) subtracted by the low rate of 3 g N pot^{-1} (N_{FB}); multiplied by 100 ((N_T-N_B/N_{FT}-N_{FB})*100).

SAS 9.2 was used for statistical analysis of data (100 SAS Campus Dr. Cary, NC 27513). The Glimmix procedure was used to obtain statistical data for N concentration, N content, biomass, height, and diameter for comparing of means across species across treatment using fixed effect, slice effect of species, and Tukey group. JMP Pro 10 (SAS software) was used for statistical analysis of all other data. Analysis of variance was used to obtain fixed effect and Tukey group.

Results and Discussion

Plant Growth

At the initiation of the study, baseline plant growth data are found in Table 2-1. There were small differences in diameters of trees among species but height varied considerably, especially between the *Acer rubrum* which was very short in comparison to the other two species. Root:shoot ratios varied considerably for *Cedrus deodara*, *Tilia cordata*, and *Acer rubrum*, respectively. There was a high Coefficient of Variation (CV) in root biomass and root:shoot ratio noted.

Tree diameter increased significantly with increasing N rates in *Acer rubrum* with a maximum occurring at 18 g N pot^{-1} (Table 2-2). Diameter in the *Tilia cordata* species only responded significantly to the 9 g N pot^{-1} rate while there was a general increasing trend but no significant response to N in *Cedrus deodara*. The magnitude of response to N from 3 to 9 g N pot^{-1} was much greater in the *Tilia cordata* species (104%) as compared to the *Acer rubrum* (35%).

As with diameter, similar results were attained in tree height among the tree species. No response was seen in *Cedrus deodara*. Tree height increased significantly with increasing N rates in *Acer rubrum* with a maximum again occurring at 18 g N pot^{-1} (Table 2-2). Response to N in the *Tilia cordata* was limited with highest height at the 27 g N pot^{-1} but significant increases did not occur beyond the 9 g N pot^{-1} rate. Much greater response in height again was seen when N was increased from 3 to 9 g N pot^{-1} in the *Tilia cordata* species (116%) as compared to the *Acer rubrum* (27%).

Given the height and diameter findings, it was not surprising to find similar results in tree biomass (Table 2-2). There was no overall response in *Cedrus deodara* but there was a general biomass trend with increasing N rate. Biomass was the greatest in *Acer rubrum*, with biomass increasing to the maximum rate of 18 g N pot^{-1}; whereas *Tilia cordata* biomass was maximized at 9 g N pot^{-1}. *Acer rubrum* generally had the highest shoot biomassof the three species, with the greatest biomass occurring at 27 g N pot^{-1} (Figure 2-1). Also *Acer rubrum* had a significant increase in leaf and shoot tissue biomass. Within tissue types, *Cedrus deodara* biomass showed no response with increasing N rate. *Tilia cordata* had the lowest

leaf biomass in comparison to the other two species, with the majority of the biomass observed in the shoot and root tissues.

There was a significant decline in root:shoot in *Tilia cordata* as N increased from 3 to 9 g N pot^{-1} (Figure 2-2). Root:shoot ratios generally declined in all tree species with increasing N rate, but it was not significant. In comparing species, *Tilia cordata* was not lower than 0.9. The magnitude of decline ranged from 1.53 at 3 g N pot^{-1} to 1.0 at 9 g N pot^{-1}, followed by an upward trend in the *Tilia cordata* species. The ratio for *Cedrus deodara* and *Acer rubrum* both had mean root:shoot ratios less than 1.0 in all treatments. This lead to a significant species effect. *Cedrus deodara's* highest root:shoot ratio was 0.57 at 3g N pot^{-1}, decreasing to 0.4 at the highest N rate. *Acer rubrum's* highest root:shoot ratio was 0.58 at 3 and 9 g N pot^{-1}, which declined to 0.41 at 27 g N pot^{-1}. There was also a significant N rate effect noted at 3 g N pot^{-1}.

Tissue

At the beginning of the experiment, N concentration in the shoot and root tissues was > 1% N in both *Acer rubrum and Tilia cordata* (Table 2-1). Both *Acer rubrum and Tilia cordata* were dormant and had no leaf tissue at the time of this measurement. In contrast, *Cedrus deodara* was well below 1% N concentration in both the shoot and root tissues; shoot tissue included leaf tissue in *Cedrus deodara*. Separation of leaf tissue did not occur due to dormancy of the other two tree species; this was checking baseline N. The greatest N concentration was detected in *Acer rubrum* even though these were the smallest trees upon arrival.

Tree root N concentration increased with increasing N rates in *Acer rubrum* and *Tilia cordata*, although the response above 9 g N pot^{-1} in the *Acer rubrum* species was not significant (Table 2-3). There was no significant effect in *Cedrus deodara*, the lowest root N concentrations (less than 1.0%) were found in this species. Root N concentration was the greatest in *Tilia cordata*, where N concentration increased to the maximum rate of 27 g N pot^{-1}.

Tree shoot N was comprised of both bud and stem (woody) tissue. Shoot tissue N concentration increased significantly with increasing N rates in *Tilia cordata* but there was no effect on shoot N concentration in *Acer rubrum* and *Cedrus deodara* (Table 2-3). Shoot N

concentration was the greatest in *Tilia cordata* where N concentration increased to the maximum rate of 27 g N pot^{-1}. The shoot N concentration was two to three times greater in *Tilia cordata* compared to shoot N in *Acer rubrum* and *Cedrus deodara*. The high N concentration in the shoot of *Tilia cordata* is very abnormal for most tree species (Cober, 1997).

For each species, there was no significant response in leaf N concentration with increasing N (Table 2-3). Also in the leaf tissue, there was no interaction effect. Within the species leaf N concentration was the greatest in *Tilia cordata*, being above 2% at 3 g N pot^{-1}. This could be explained because leaf biomass was the lowest at 3 g N pot^{-1} in *Tilia cordata* (Figure 2-1). Leaf drop was observed in the lower N treatments in *Tilia cordata*, especially at 3 g N pot^{-1} but also at 9 g N pot^{-1}. The high N concentration detected in the 3 g N pot^{-1} rate in *Tilia cordata* was likely due to only a few immature leaves per tree to evaluate. N concentration in immature leaves tends to be higher than in mature leaves; concentrations of N tends to be decrease as leaves mature (Barker and Pilbeam, 2007). Increasing N rates in *Tilia cordata* increased leaf biomass and the number of mature leaves. In comparison to known critical ranges, leaf tissue N fell below established ranges, with the exception of *Cedrus deodara.* No established critical ranges for *Tilia cordata* could be found. *Cedrus deodara* leaf N concentrations were within the sufficiency ranges for conifer nursery stock, falling between 1.3-3.5 % N, but were considered high in comparison to N surveys of mature *Cedrus deodara* at 1.0-1.4 % N (Landis et al., 2010; Perry and Hickman, 2001). *Acer rubrum* leaf N concentration was noticeably lower than the N critical range for mature established *Acer* species of 2.0-3.4 % N (Perry and Hickman, 2001). However, these ranges may not be adequate due to multiple factors including age, season, growing conditions, and species (Dreistadt et al., 2001). All tissue samples were collected early in the fall, which may have influenced N concentration, especially in the leaf tissue. As the growing season progressed, N concentration could be diluted by rapid growth from the start to the end of the growing season (Insley et al., 1981).

Distribution of N between leaf, shoot, and roots was significantly different among tree species (Figure 2-3). There was no response observed in N distribution as N rate increased with species, but there was a significant species effect noted in all tissues. In *Acer*

rubrum, the majority of N (~75%) was found in the leaf and root tissue, with leaf levels ranging from 39-45 % N and root levels ranging from 30-37 % N, leaving the remaining 21-24 % N in the shoot tissue. *Cedrus deodara* contained the majority of N in the foliage, with 59-64 % N in the leaf tissue, while only retaining 20-26 % in the root tissue and 14-16 % N in the shoot tissue. *Tilia cordata* differed from the other two tree species in that > 80 % of the N resided in the woody tissue (shoot and root), with 36-40 % N in the shoot and 45-59 % N in the root tissue, respectively. The leaf tissue retained 5-17 % of the N in *Tilia cordata* whereas the shoot tissue was the greatest sink, leaf biomass equaled a small portion in *Tilia cordata* in comparison to *Acer rubrum* and *Cedrus deodara* (Figure 2-1). The majority of the N in *Tilia cordata* was located in the shoot and root tissues, with the shoot tissue seeming to be a sink for N in comparison to the other two trees species in this experiment. *Acer rubrum* and *Cedrus deodara* exhibited no significances in shoot tissue N with increasing N rates. High N distribution in the shoot of *Tilia cordata* was possibly associated with the outer layers of cambium, phloem and periderm where storage or photosynthesis could be expected to occur in some young trees (Pfanz et al., 2002).

There was no significant decrease in NUE with increasing N rate among three tree species (Figure 2-4). However, there was a trend noted as N rates increased, NUE decreased in all species. There was a significant species effect noted and N rate effect had a p-value of 0.0538. *Tilia cordata* had the highest NUE in comparison to the other two species. *Acer rubrum* had a slightly low NUE than *Tilia cordata*, although there was no statistical difference. *Cedrus deodara* had the lowest NUE among the species and was significantly lower in comparison to *Tilia cordata* but not *Acer rubrum*. The reduction in NUE would be expected as N rates increased. However, *Cedrus deodara* demonstrated that it was not as well adapted to uptake high N rates in comparison to the other two species.

Pine Bark Soilless Media

As accessed by the pour-through method, inorganic N (IN-NO_3^- and NH_4^+) in leachate increased with increasing N rates in all tree species at the latter two sampling periods (Table 2-4). There was a significant time, species, rate, and interaction effect noted in both NO_3^- and NH_4^+ leachate; there was no pot effect noted. Samples taken in April before application of fertilizer resulted in similar IN levels. In May, samples collection ranged from

38 to 45 days after fertilization, resulting in increased levels of IN. The highest IN levels were observed in *Cedrus deodara,* while *Tilia cordata* had the lowest levels. Samples collected in August ranged from 125 to 132 days after fertilization. In August, *Tilia cordata* had the greatest IN levels within media leachate, while *Acer rubrum* had the lowest levels. In *Tilia cordata* IN levels in the August collection period should have decreased due to the capsulated fertilizer being exhausted as was seen in the *Cedrus deodara* and *Acer rubrum*. Both fertilizer labels stated at temperatures of 26°C, the products would supply N for approximately 180 days; however, release rates of the capsulated fertilizers are affected by temperature, with higher temperature increasing N release rates (Merhaut et al., 2013). As a result, the high IN levels in the August collection period for *Tilia cordata* may be due to some variations in collection. It is possible that the high sample numbers, large container size, and timing of sampling, possibly influenced results (Bilderback, 2001). Sample numbers were large and led to long sampling times. The NUE was greater in *Tilia cordata* versus *Cedrus deodara,* which could indicate higher IN loss in *Cedrus deodara* (Figure 2-4). The saturated media extract method for measurement of IN may have given a more uniform result; it also would have required more time and damaged the sample root system. In comparison of leachate after fertilization, NO_3^- levels tended to increase with time, where NH_4^+ tended to decrease, leading to the increase in NO_3^-:NH_4^+ ratio in leachate over time. The NO_3^-:NH_4^+ varied greatly with increasing N rates in the latter two sampling periods. The highest NO_3^-:NH_4^+ ratios were observed in August, with ratios ranging from 5.2-18.6; with 9 to 18 g N pot^{-1} containing the highest NO_3^-:NH_4^+ ratios. However, NH_4^+ and urea made up roughly 95 % of N added in 27 g N pot^{-1}. Nitrification of NH_4^+ probably was the key factor in the increase in NO_3^- to NH_4^+ ratios (Elliott & Lang, 1997).

As N rates increased in May and August, leachate pH deceased, even with the addition of lime at the beginning of the experiment (Figure 2-5). In August the lowest pH values were associated with the highest N fertilizer treatment of 27 g N pot^{-1}. *Cedrus deodara* had the lowest pH of 5.4 observed in August leachate collection, followed by pH 5.7 in *Tilia cordata* and 5.8 in *Acer rubrum*. Nitrogen contributed to greatly decreasing substrate pH with increased N rates, due to plant uptake of NH_4^+ and nitrification (Foy et al, 1978; Elliott and Lang, 1997).

In May and August as N rate increased there was a correlated increase in EC. The highest EC values were detected in the 27 g N pot^{-1} fertilizer treatment (Figure 2-6); however, highest EC values were observed in May for both *Acer rubrum* and *Cedrus deodara* and August for *Tilia cordata*. With increasing N rates in May and August, EC increases significant, except in *Cedrus deodara* in August as N rates increased above 9 g N pot^{-1} there was no significant increase. EC tended to decrease in the August collection period for *Acer rubrum* and *Cedrus deodara*, but increased for *Tilia cordata* for each collection period with the highest EC observed in August. In August, nutrient ions from the fertilizer capsules should have been exhausted. Note that EC may not be directly correlated with N rate increases; nonessential salts like sodium as well as the increase decomposition of the soil-less media from higher N rates and low pH could contribute to EC increases (Merhaut et al., 2013).

Conclusion

Tree species responded differently to increasing N rates, which suggests differences in the ability to uptake, store, and utilize N. *Tilia cordata* showed the best capability to load N with significant increases in N concentration in root and shoot tissues. *Acer rubrum* showed a continuous increase in biomass with increasing N rate and the ability to load N with significant increase in N concentration in the root tissue. *Cedrus deodara* had little changes within any parameter with increase in N. *Tilia cordata* showed one difference in comparison to the other two tree species; that is, the ability to buildup high N levels in the shoot tissue, where normally trees contain the majority of their N in the leaves. *Acer* and *Tilia* tree species are considered high N accumulators (Ducnuigeen et al., 1997 and T.R. Crow, 2003). *Cedrus deodara* was a minimum N consumer in this study. In most cases deciduous trees have a greater demand for N than do conifers (Cole and Rapp, 1981). Higher levels of granular SRN can be used for loading N with somewhat less NUE for reaching optimal levels of N in the tissue as well as increasing growth in *Tilia cordata* and *Acer rubrum*. With the addition of N as NH_4^+, one must consider the decrease in pH and the addition of more lime to counteract the acidity produced in the fertilizer. Also, further focus should be applied to constant N rates versus exponential N rates, and finding species

response limitations to the applied N with a center of attention on plant age where N concentration can decrease as plant age increases (Barker and Pilbeam, 2007). Our goal should be optimizing health and growth of each container grown species.

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Table 2-1. Plant growth parameter and N concentration prior to N treatment.

Tree	Root[1]	Shoot[1]	Diameter[1]	Height[1]	Root[1]	Shoot[1]	Root:Shoot
	--------- *% N* ---------		*mm*	*cm*	---------- *g* ----------		
Tilia cordata	1.16	1.20	6.1	52.1	3.08	3.46	0.89
Acer rubrum	1.47	1.27	4.4	14.9	1.06	0.82	1.25
Cedrus Deodara[2]	0.63	0.80	5.0	44.4	5.82	8.90	0.66
Coefficient of Variation (CV)							
----------%----------							
Tilia cordata	6	26	5	3	16	12	7
Acer rubrum	10	8	8	8	54	16	45
Cedrus Deodara[2]	5	11	9	3	10	19	10

[1]Average values obtained from five trees from each species.
[2]Leaf tissue included in *Cedrus Deodara* analysis.

Table 2-2. Effects of N rates on tree diameter, height, and biomass.

Trees	N rate	Diameter[1]	Height[1]	Biomass[1]
Tilia cordata	*g pot^{-1}*	*mm*	*cm*	*g*
	3	2.66a	19.8a	30.5a
	9	8.39b	73.9b	74.6ab
	18	8.91b	74.5b	115.1b
	27	8.94b	81.5b	103.5b
	p	< 0.0001*	< 0.0001*	0.0434*
Acer rubrum				
	3	5.69a	92.1a	69.9ab
	9	8.14b	121.0b	112.2bc
	18	9.83c	136.1c	146.2cd
	27	9.80c	136.0c	195.8d
	p	< 0.0001*	< 0.0001*	0.0018*
Cedrus deodara				
	3	3.41a	43.8a	74.5a
	9	4.46a	47.1a	102.6a
	18	4.90a	44.9a	84.6a
	27	5.01a	48.9a	94.5a
	p	0.1300	0.9031	0.8215
Fixed Effects				
N Rate		< 0.0001*	< 0.0001*	0.0013*
Species		< 0.0001*	< 0.0001*	0.005*
Interaction		< 0.0001*	< 0.0001*	0.204

[1] Means within columns with the same letter(s) are not significantly different at

Table 2-3. Effects of N rates on leaf, root, and stem tissue N concentration.

Trees	N rate	N Leaf[1]	N Root[1]	N Shoot[1]
Tilia cordata	*g pot^{-1}*	---------- % ----------		
	3	2.66a	1.11a	1.06a
	9	2.21a	1.35bc	1.42b
	18	2.35a	1.49c	1.47b
	27	2.32a	1.74d	1.79c
	p	0.1949	< 0.0001*	< 0.0001*
Acer rubrum				
	3	1.58a	0.95a	0.58a
	9	1.81a	1.20b	0.69a
	18	1.83a	1.28b	0.64a
	27	1.86a	1.19b	0.61a
	p	0.3718	0.0002*	0.6562
Cedrus deodara				
	3	1.61a	0.71a	0.53a
	9	1.72a	0.75a	0.55a
	18	1.56a	0.78a	0.54a
	27	1.66a	0.82a	0.53a
	p	0.7218	0.5147	0.9978
Fixed Effects				
N Rate		0.9607	< 0.0001*	0.0002*
Species		< 0.0001*	< 0.0001*	< 0.0001*
Interaction		0.2717	< 0.0001*	< 0.0001*

[1] Means within columns with the same letter(s) are not significantly different at

Table 2-4. Nitrogen rate effects on inorganic N concentration in leachate

		April 2 - 9			May 21 - 28			August 16 - 23		
Tree	N rate	NO_3^-	NH_4^+	NO_3^-:NH_4^+	NO_3^-	NH_4^+	NO_3^-:NH_4^+	NO_3^-	NH_4^+	NO_3^-:NH_4^+
Tilia cordata	*g pot^{-1}*	---- *mg L^{-1}* ----			---- *mg L^{-1}* ----			---- *mg L^{-1}* ----		
	3	0.72a	0.70a	1.0a	0.68a	0.39a	1.8a	7.54a	0.59a	12.9b
	9	0.75a	0.68a	1.1a	5.00ab	1.18a	4.9a	10.38a	0.74a	12.7ab
	18	0.76a	0.68a	1.1a	11.26bc	2.71a	4.4a	49.63b	3.97b	16.8b
	27	0.75a	0.68a	1.1a	18.94c	5.86b	3.5a	82.88c	13.56c	8.2a
Acer rubrum										
	3	0.71a	0.69a	1.0a	0.90a	0.30a	2.9a	2.16a	0.39a	5.5a
	9	0.70a	0.67a	1.0a	7.48ab	0.88a	9.2c	6.64ab	0.71a	10.4bc
	18	0.68a	0.63a	1.1a	16.25bc	2.86a	5.9abc	14.20bc	1.06a	14.4c
	27	0.69a	0.63a	1.1a	26.15c	7.10b	3.9ab	20.83c	3.15a	7.5ab
Cedrus deodara										
	3	0.71a	0.71a	1.0a	0.93a	0.35a	2.7a	2.49a	0.39a	8.1ab
	9	0.71a	0.70a	1.0a	8.40ab	1.28a	9.4b	18.18b	1.74a	18.6c
	18	0.71a	0.70a	1.0a	16.79bc	6.30b	3.1a	23.66b	2.81a	13.4bc
	27	0.71a	0.70a	1.0a	31.98d	13.84c	2.8a	26.82b	6.31b	5.2a

Fixed Effects	NO_3^-	NH_4^+	NO_3^-:NH_4^+
Time	< 0.0001*	< 0.0001*	< 0.0001*
Species	< 0.0001*	< 0.0001*	0.4115
N Rate	< 0.0001*	< 0.0001*	< 0.0001*
Interaction	< 0.0001*	< 0.0001*	0.0007*
Pot	0.0761	0.1758	0.3796

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

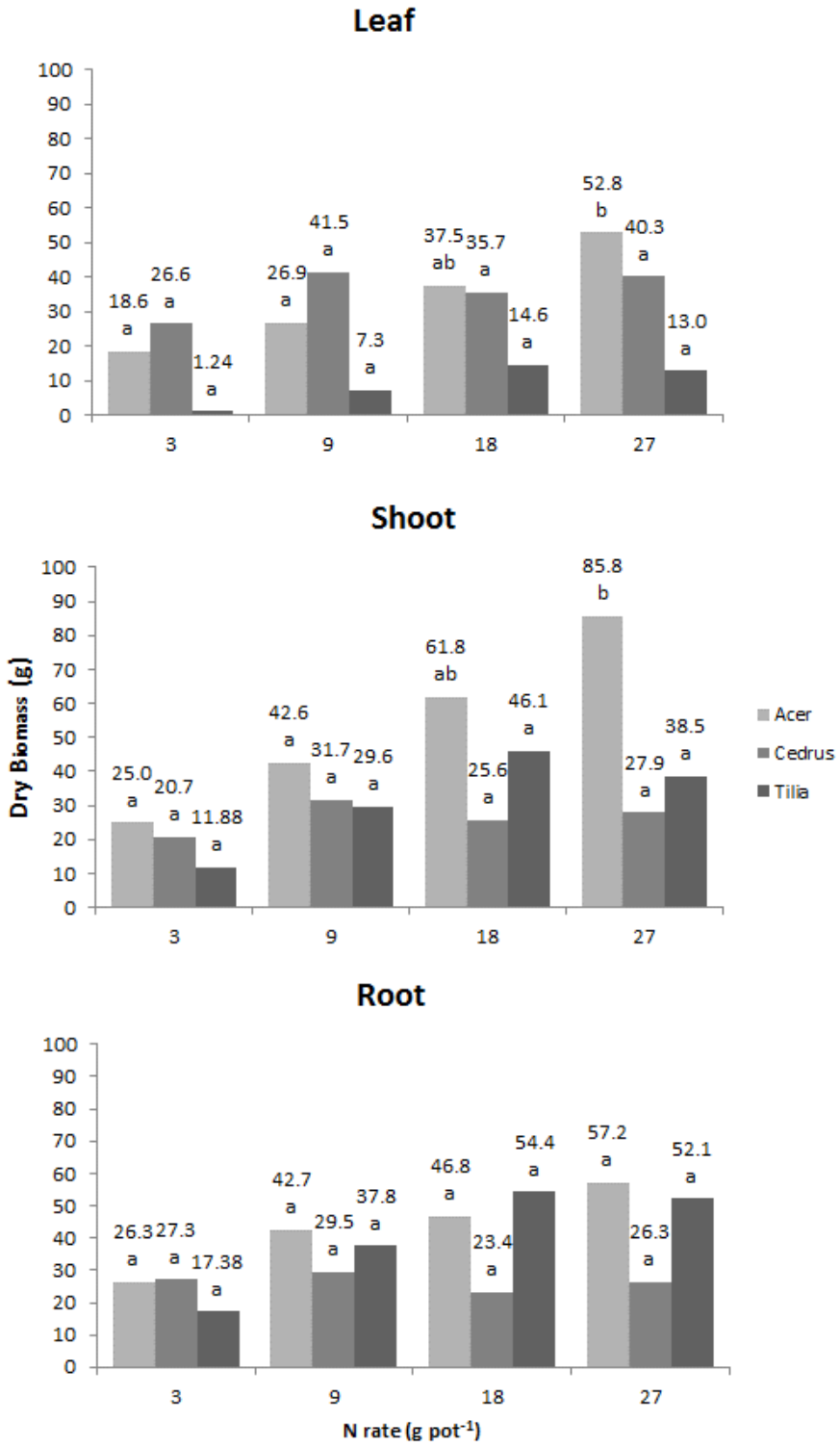


Figure 2-1. The effects of N rates on biomass yield in leaf, woody shoot, and root tissues. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

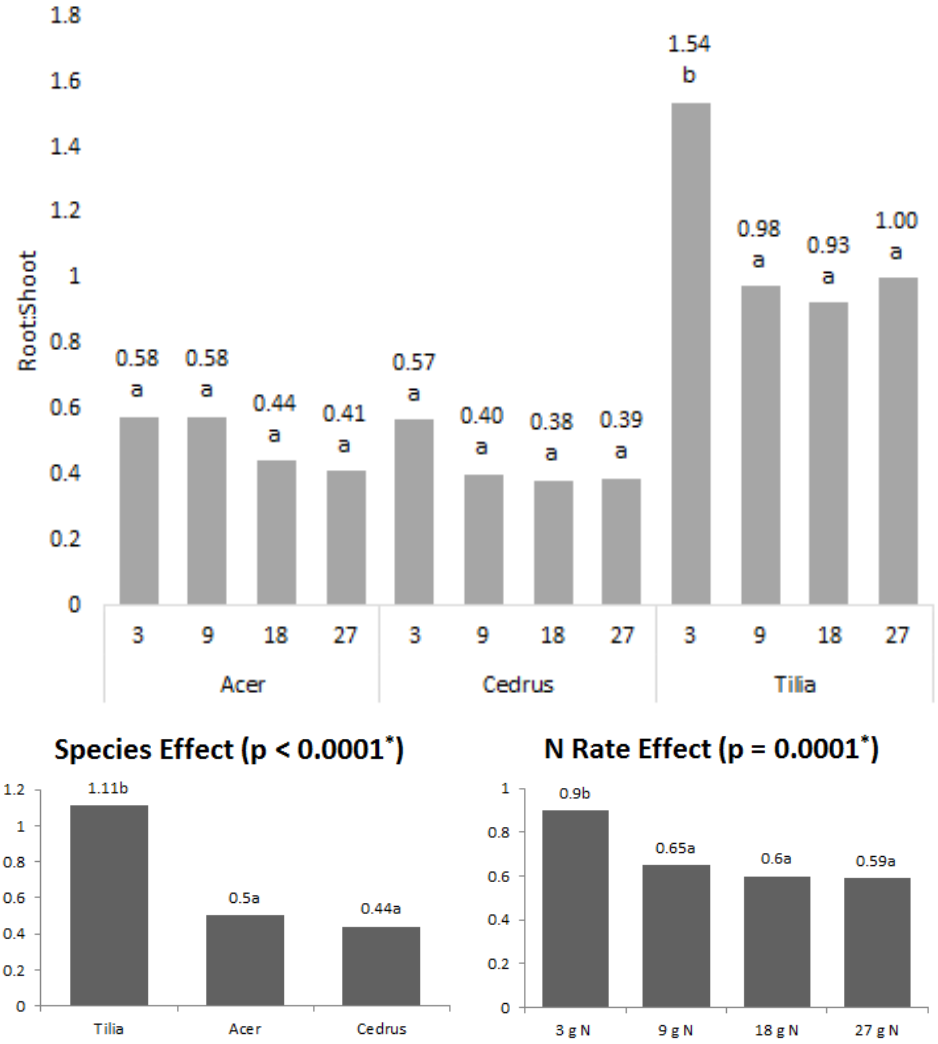
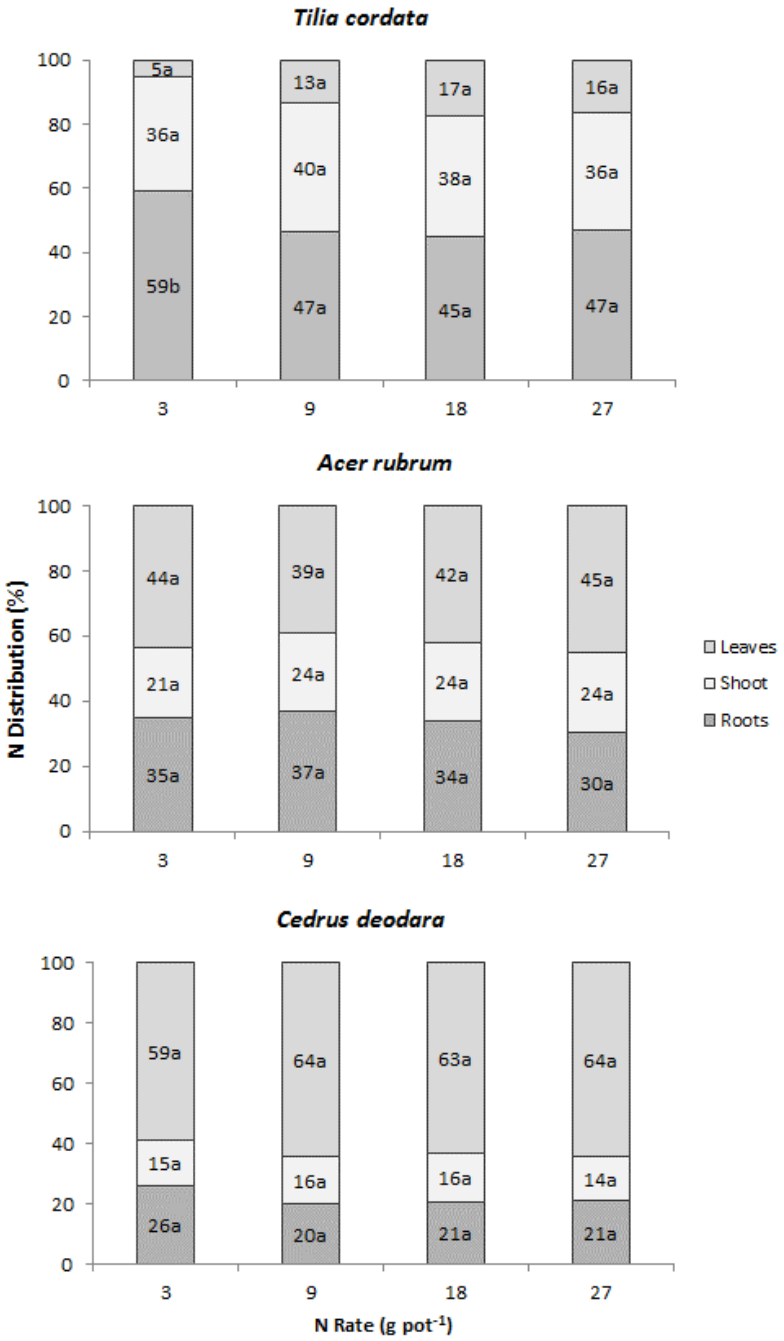


Figure 2-2. Root to shoot ratio of tissue biomass with increasing N rates; alone with N rate effect and species effect. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.



Leaf N: Species effect $p < 0.0001^{*}$; N rate effect $p = 0.1095$
Shoot N: Species effect $p < 0.0001^{*}$; N rate effect $p = 0.2887$
Root N: Species effect $p < 0.0001^{*}$; N rate effect $p = 0.0286^{*}$

Figure 2-3. The effects of N rates on the distribution of N in leaf (top), shoot (middle), and root (bottom) tissues. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

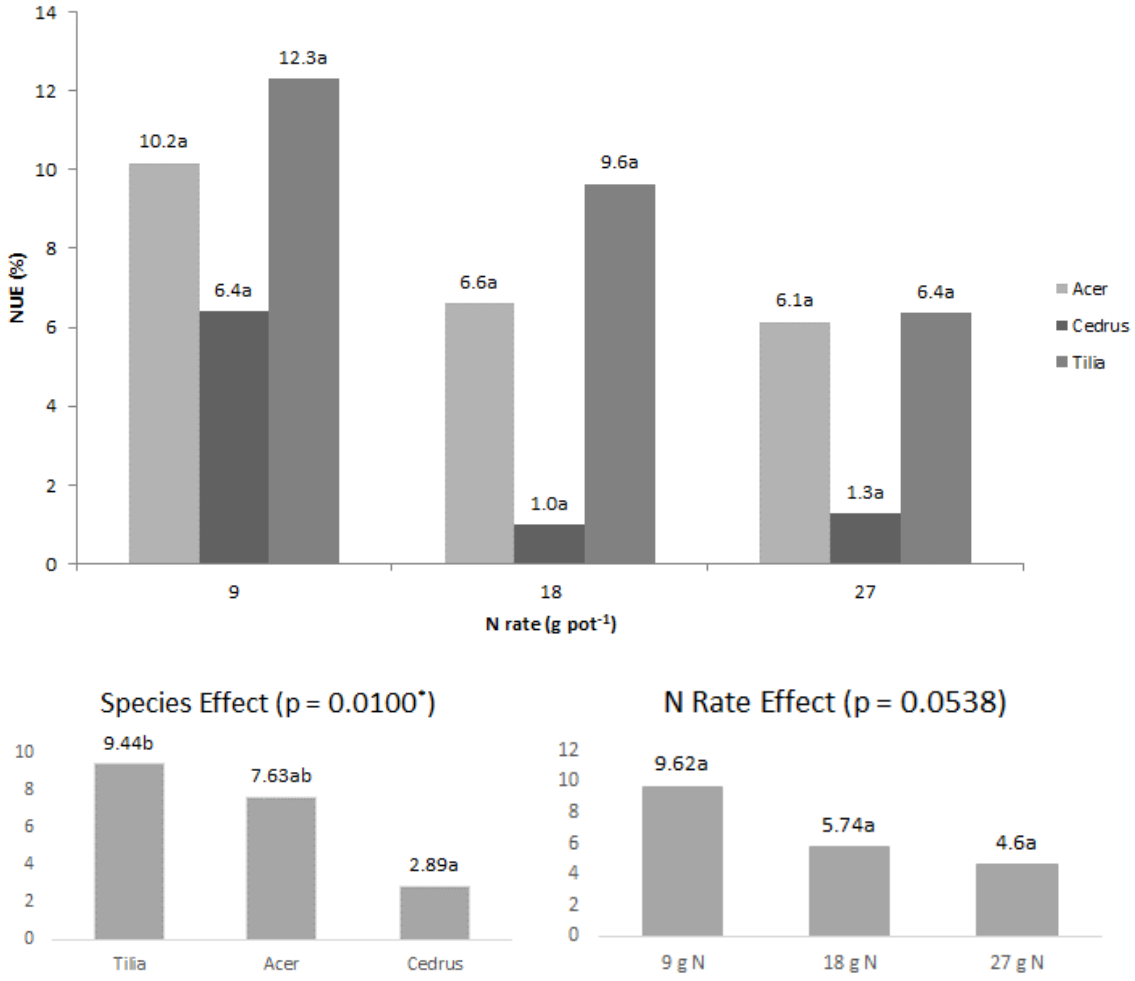


Figure 2-4. N rate effects on N use efficiency (NUE) in three tree species. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

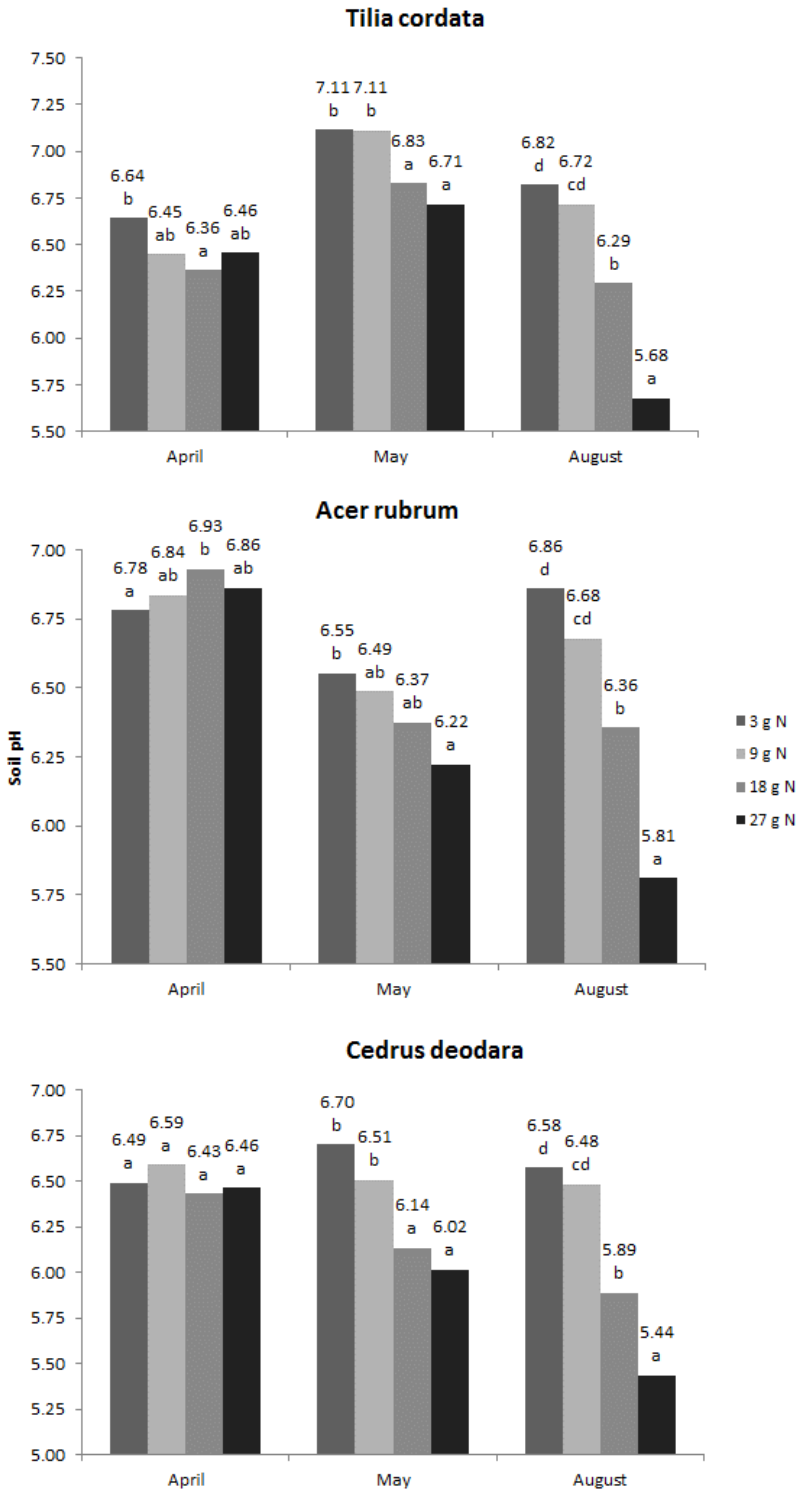


Figure 2-5. Media leachate pH changes throughout the duration of plant development, with increasing N rates. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

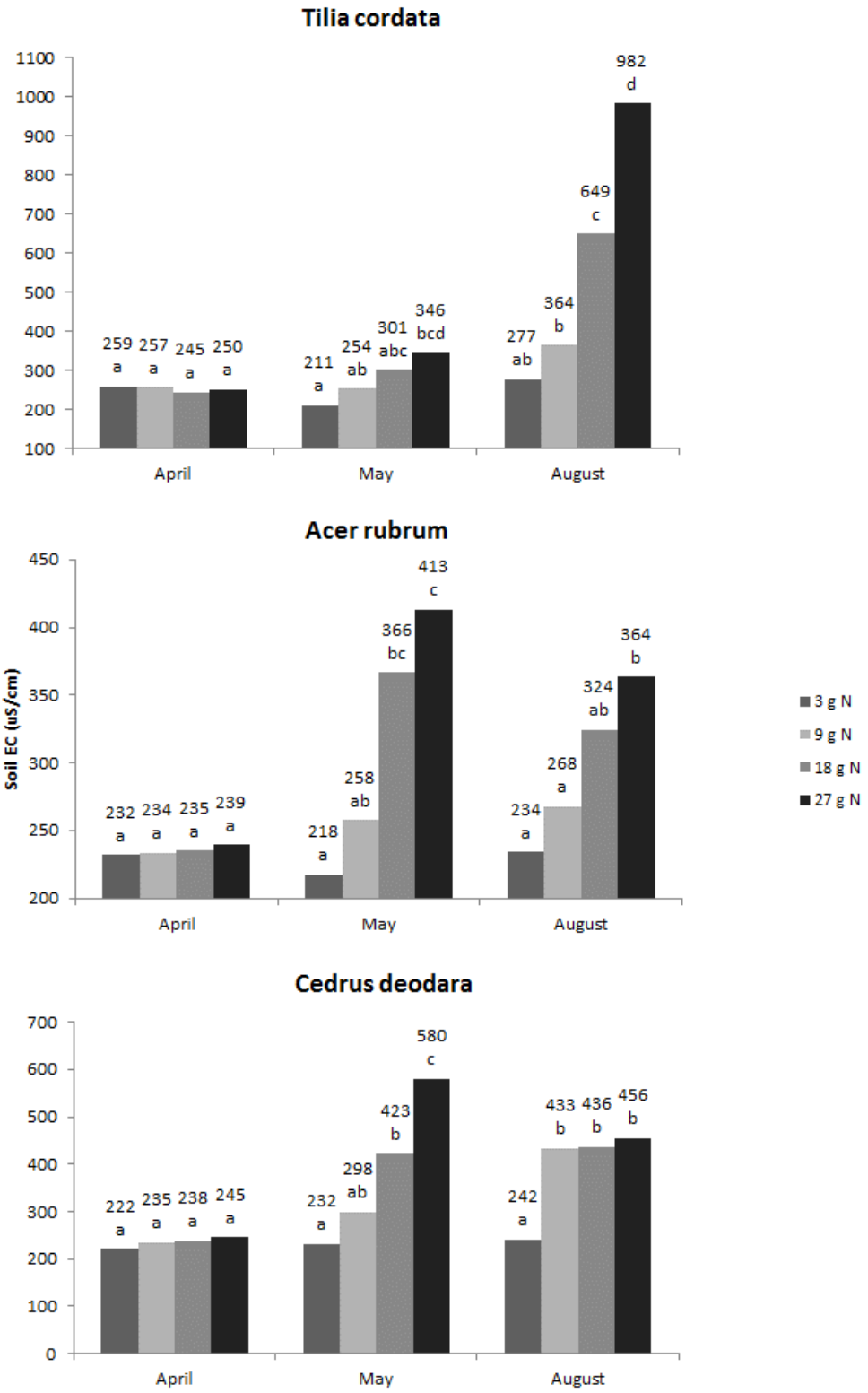


Figure 2-6. Media leachate EC (soluble salts) changes throughout the duration of plant development, with increasing N rates. Means with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison.

APPENDICES

Table A-1. Effects of soil pH on *V. vinifera* height and biomass in 2011.

Target Soil pH	Height[1] --------*cm*--------	Biomass[1] --------*g*-------
4	48.8c	30.2c
5	52.2bc	38.1bc
6	72.0ab	44.6ab
7	90.6a	50.6a
p	0.0001*	0.0004*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table A-2. Effects of soil pH on *V. vinifera* leaf tissue nutrient concentrations in 2011.

Target Soil pH	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
	---------- % ----------					
4	2.28a	0.17a	2.21a	0.61c	0.29a	0.35a
5	1.86b	0.15a	1.60b	0.78c	0.27a	0.29b
6	1.75b	0.15a	1.36b	1.03b	0.24a	0.28b
7	1.59b	0.17a	1.28b	1.54a	0.24a	0.25b
p	0.0009*	0.1826	0.0003*	< 0.0001*	0.0351*	0.0007*
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	
	---------- *mg kg^{-1}* ----------					
4	215a	144a	35.8a	7.2a	47.4a	
5	193a	175a	36.0a	5.9b	38.5b	
6	175a	158a	34.7a	5.7b	27.6c	
7	179a	136a	37.9a	5.5b	22.5c	
p	0.1081	0.2010	0.9003	0.0079*	< 0.0001*	

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment

Table A-3. Effects of soil pH on *V. vinifera* root tissue nutrient concentrations in 2011.

Target	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
Soil pH	---------- % ----------					
4	1.63a	0.15a	0.43a	0.20c	0.058a	0.20ab
5	1.20b	0.12b	0.40a	0.30bc	0.050ab	0.16a
6	1.18b	0.14ab	0.56a	0.38b	0.048b	0.24b
7	1.03b	0.13ab	0.57a	0.55a	0.054ab	0.22b
p	< 0.0005*	0.0482*	0.0211*	< 0.0001*	0.0484*	0.0088*
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	
	---------- *mg kg^{-1}* ----------					
4	717a	48.8a	43.2a	13.8a	8.7a	
5	460b	34.0b	39.0ab	7.5b	9.2a	
6	597ab	31.8b	35.5ab	9.4ab	8.5a	
7	558ab	19.4c	30.7b	8.8ab	9.0a	
p	0.0093*	< 0.0001*	0.0175*	0.0457*	0.5154	

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table A-4. Mehlich 3 soil test results of soil pH treatments at end of study in 2011.

Target	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
Soil pH	---------- *mg L^{-1}* ----------				
4	29.2a	61.8a	87.2c	17.3a	38.4a
5	28.7a	57.9ab	108.0c	17.3a	34.3b
6	27.4ab	51.6bc	183.6b	17.3a	31.8b
7	25.4b	44.6c	338.0a	16.8a	31.3b
p	0.0177*	< 0.0001*	< 0.0001*	0.6150	< 0.0001*
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	Al[1]
	---------- *mg L^{-1}* ----------				
4	224a	1.18a	0.94a	0.56a	874ab
5	223a	1.18a	0.94a	0.50a	877a
6	217a	1.08ab	0.90a	0.50a	853ab
7	214a	0.98b	0.88a	0.54a	830b
p	< 0.5760	< 0.0001*	< 0.1219	< 0.0615	0.0371*

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment.

Table A-5. Effects of soil pH on *V. vinifera* stem tissue nutrient concentrations in 2012.

Target Soil pH	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
	---------- % ----------					
4	0.70a	0.06b	0.47b	0.42b	0.11a	0.10a
5	0.67a	0.07b	0.68a	0.58ab	0.11a	0.10a
6	0.71a	0.10a	0.76a	0.67a	0.10a	0.09a
7	0.75a	0.10a	0.75a	0.65a	0.09a	0.07a
p	0.3622	< 0.0001*	0.0002*	0.0020*	0.0969	0.0747
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	Al[1]
	---------- *mg kg^{-1}* ----------					
4	88.7a	140.2ab	79.1a	10.5a	19.8a	37.8a
5	118.7a	188.8a	81.6a	8.4a	21.3a	44.3a
6	82.6a	124.6b	61.7ab	10.2a	25.2a	25.7a
7	70.0a	53.2c	44.4b	10.4a	23.1a	25.9a
p	0.1041	< 0.0001*	0.0008*	0.5152	0.1131	0.3805

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment

Table A-6. Effects of soil pH on *V. vinifera* stem tissue nutrient concentrations in 2011.

Target Soil pH	N[1]	P[1]	K[1]	Ca[1]	Mg[1]	S[1]
	---------- % ----------					
4	0.51a	0.07a	0.34ab	0.48b	0.094ab	0.07a
5	0.46a	0.07a	0.35a	0.55ab	0.100a	0.06a
6	0.45a	0.06a	0.29b	0.54ab	0.084bc	0.06a
7	0.47a	0.07a	0.33ab	0.56a	0.080c	0.06a
p	0.5774	0.4231	0.0377*	0.0446	0.0009*	0.3844
	Fe[1]	Mn[1]	Zn[1]	Cu[1]	B[1]	
	---------- *mg kg^{-1}* ----------					
4	177.9a	59.3a	61.3a	7.1a	15.9a	
5	115.2a	59.4a	45.7a	6.4a	16.1a	
6	74.8a	46.8b	37.7a	6.8a	13.9a	
7	123.6a	38.8b	35.3a	5.7a	14.2a	
p	0.4979	0.0004*	0.5259	0.5551	0.06	

[1] Means within columns with the same letter(s) are not significantly different at $p \leq 0.05$ by Tukey's multiple pairwise comparison of treatment