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GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES AND CONIFERS IN RESPONSE TO FERTILIZER AND MEDIA IN POT-IN-POT PRODUCTION SYSTEMS FOR NOTHERN CLIMATES

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GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES AND CONIFERS IN RESPONSE TO FERTILIZER AND MEDIA IN POT-IN-POT PRODUCTION SYSTEMS FOR NOTHERN CLIMATES

By

Wendy Sue Klooster

A THESIS

Submitted to
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# ABSTRACT

## GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES AND CONIFERS IN RESPONSE TO FERTILIZER AND MEDIA FOR POT-IN-POT PRODUCTION SYSTEMS IN NORTHERN CLIMATES

By

Wendy Sue Klooster

Container production is increasing relative to balled-and-burlapped (B&B) production throughout the US. Pot-in-pot (PIP) production is a form of container production that combines the benefits of conventional above-ground container production with those of field production. The goal of this research was to develop guidelines for fertilizer application and media type to improve PIP production for growers in the upper Midwest. The project consisted of two studies: 1) examining fertilizer effects on seven taxa of deciduous shade tree, and 2) examining effects of fertilizer and media on four conifer species. Growth and physiological responses were measured throughout two growing seasons. From the first study, we found that fertilizer addition increased growth of shade trees largely by increasing total leaf area per tree. Suggested fertilizer rates are consistent with previous recommendations of adding 6.33 $g \cdot L^{-1}$ container. Increased leaf area resulted in increased water use and reduced stomatal conductance, which likely offset any increased photosynthetic efficiency due to fertilization. Results from the second study indicate that maximum growth of conifers occurs with fertilizer addition between 3.92 and 7.84 $g \cdot L^{-1}$ container. Furthermore, for container substrates consisting of pine bark and peat moss, a single mix of 80% bark to 20% peat moss is appropriate for all the species tested.

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# LITERATURE REVIEW

## Introduction

In the upper midwestern US, sales of container nursery material, including conifers and deciduous shade trees, have been increasing relative to balled and burlapped (B&B) material (NASS, 2007a). In many parts of the country, container production of trees and shrubs has even surpassed field production (NASS, 2007a, 2007b; Neal, 2004). Pot-in-pot (PIP) production is a form of container production that combines the benefits of both field and conventional above-ground container (AGC) production. This system was developed in Virginia in the late 1980s, and much of the subsequent research has been performed in the southern US, particularly in Georgia by Ruter and his coworkers (Ruter, 1994, 1998a, 1999b). A primary concern in southern nursery production is moderating root-zone temperatures during the hot summers (Mathers, 2003); in contrast, northern growers have begun adopting the PIP production system as a way of protecting roots during the cold winter months (Mathers, 2003; Neal, 2004).

PIP production is well-suited to northern climates; however, since root-zone temperatures affect the rates of nutrient and water uptake, the milder summers and colder winters may require different fertilizer, media, and irrigation recommendations for the northern US than for the South. In addition, physiological responses to PIP, which will be discussed in more detail later, differ between southern and northern climates (Neal, 2004). A greater knowledge of how trees grown in PIP production systems, particularly in northern climates, utilize resource inputs will enable us to provide growers throughout the upper Midwest with improved management-practice recommendations.

Many growers in Michigan and the Midwest have already begun transitioning from field and AGC production to PIP production; however, a number of them experienced difficulties when the cultural factors they had selected did not result in a satisfactory finished product (Jill O'Donnell, Michigan Christmas Tree Integrated Crop Management Specialist, personal communication). This study was initiated in response to questions from the growers and industry representatives on how to optimize the PIP production system to suit their needs. In talking with them, we identified two separate issues: 1) some growers were focusing on rapid production of deciduous shade trees for use as replacements for trees lost to diseases or invasive pests, specifically the emerald ash borer; and 2) other growers were more interested in production of conifers for use in the landscape or as Christmas trees. Different production goals require different management practices. Since the cultural factors that growers have most control over are irrigation and nutrient additions, and media properties have a large influence on irrigation and nutrient management, this study was designed to compare different media combinations and fertilization levels in order to optimize productivity within the PIP production system.

The goals of our research were to accelerate nursery production, maximize grower profitability, and increase the availability of replacement trees for those lost due to invasive pests or diseases through the use of PIP production systems. Specific objectives were to: 1) determine the physiological responses to varying nutrition rates; 2) minimize environmental impact and grower expense by identifying an optimum nutrition rate; 3) determine which of three media combinations resulted in the greatest amount of growth of four conifer species; 4) develop nutrient diagnostics using indirect methods such as

SPAD or chlorophyll fluorescence measurements; and 5) use the diagnostics to develop foliar nutrient concentration guidelines to allow growers to conveniently monitor the health of their trees.

In this literature review I will first discuss the general characteristics of PIP production that pertain to both deciduous shade and conifer trees. I will then examine cultural factors specific to deciduous shade tree production and landscape conifer production separately. Finally, I will discuss the physiological responses of deciduous shade and conifer trees to various cultural factors.

**General Considerations of PIP Production**

*Comparison with Field Production*

Field production has long been a dominant form of production for both deciduous shade and conifer trees (Powell et al., 1996). Some growers and landscapers still prefer field-grown trees over container material (Neal, 2004); in particular, some are hesitant to use PIP trees because they initially require more watering after transplant, and most landscapers already have the equipment to handle field-dug trees (Mark Pavelek, Container Production Manager, Wiegand's Nursery, Macomb, MI, personal communication). Nevertheless, consumers have shown increased preference for container material (Brooker et al., 2000, 2005), and PIP production, in particular, offers many advantages over in-field production.

Due to the heavy machinery required to harvest field-grown trees, the soil is compacted more than in any other type of farming; in contrast, during harvest of PIP material, root-zone compaction is essentially non-existent because of the use of container media rather than field soil (Mathers, 2000). Furthermore, when field-grown trees are harvested, a large amount of soil is lost, as much as 100 tons per acre for a five year rotation, which must be replaced for subsequent production (Pollock and Mathers, 2002); this is not a concern in container production.

In addition to soil loss, more than 90% of a field-grown tree root system may be lost during harvest, resulting in severe transplant stress and a longer acclimation process compared to container-grown trees (Gilman, 1988). The majority of roots lost are fine roots, which are responsible for most of the nutrient and water uptake (Mathers et al., 2007). In container production, total root dry weight is typically increased relative to field production, and the roots remain intact during the harvesting and transplanting process (Mathers et al., 2007).

The planting and harvesting period is greatly extended for container-grown plants compared with field-grown plants (Mathers et al., 2007; Neal, 2004). Harvesting B&B trees typically takes place in early fall and late spring, when the ground is firm but not frozen; in early spring, the ground is often too saturated, and the heavy equipment would cause excessive compaction of the soil. It is possible for container-grown plants to be potted in a greenhouse during the winter months when business is otherwise slow; they can then be harvested and shipped any time during the growing season (Mathers et al., 2007).

Growing large-caliper trees in containers reduces the labor and equipment costs compared to field production (Anslow, 2007). Since lightweight potting media is used, a tree grown in a container typically weighs less than half of what the same size tree would weigh if it were B&B; lightweight containers require fewer people and less equipment to harvest and also make the trees easier for customers to manage (Anslow, 2007).

Reducing the amount of space required for production is another way PIP systems reduce costs for nursery growers. With container production, up to eight times more plants can be grown per unit area compared to field production, since it is not necessary to leave wide rows for equipment access (Anslow, 2007).

Land quality is also less of an issue with container production compared to field production. Since PIP trees are grown in container media, they are not as affected by the native soil, and areas of land not suited for field-growing can be utilized for production (Keith Hilton, Container Production Manager, Kluck Nursery, Saginaw, MI, personal communication). For example, some sites that have poor soils can be converted to PIP production rather than requiring the addition of amendments; although it may be necessary to install drainage tile if the soil has high clay content. Furthermore, if diseases such as *Phytophthora*, a common fungus affecting ornamental plants, are introduced in a field-production site, they may cause extensive mortality and may persist long enough to cause nurseries to abandon that area of land (Kuhlman et al., 1989; Linderman and Davis, 2006).

Some growers are still apprehensive to convert to PIP production because of the high start-up cost involved with installation and additional materials required, such as two pots per plant and drainage tile. A modeling study comparing in-field, AGC, and PIP production systems confirmed that PIP had the highest initial cost; however, after one three-year production cycle, the cost of producing a finished plant was lowest for the PIP system due to less intensive cultural practices (Adrian et al., 1998). In actual nursery settings, where management practices may not be as ideal, growers have found it can take five to six years to recoup their investments, but they are more satisfied with the finished product (Anslow, 2007). The gaining popularity and acceptance of PIP production is evidenced by the development of equipment designed specifically for installation of PIP systems; some even prepare a trench, lay drainage tile and drip line, and install the pots all in one pass (Anslow, 2007).

*Comparison with Conventional Container Production*

Pot-in-pot production is a form of container production that has distinct advantages over conventional AGC production. The system retains many of the benefits of field growing to eliminate the problem of windthrow and provides increased protection for plant root-zones from extreme temperatures; and since the plants are grown in containers, they are still as lightweight and easy to manage as in AGC production.

Windthrow, containerized plants blowing over in the wind, is a common problem of AGC production. This often results in loss of potting media and granular fertilizer, which then have to be replaced, as well as the additional labor required to right plants. By sinking the container into the ground and using a second container as a 'socket pot' to provide structural support and stability, PIP production eliminates windthrow and the extra expense of replacing lost material, and allows for more efficient use of labor (Neal, 2004).

Another labor-saving benefit of PIP production is the ability to over-winter trees in the ground. This eliminates the time and labor expense of moving the trees inside a poly-house in the fall, and moving them back outside in the spring, as well as the cost of the structure. Since the containers are sunk into the ground and insulated by the surrounding soil, temperatures in PIP root-zones are consistently lower than in AGC root zones during the summer (Zhu et al., 2005), and are comparable to root-zone temperatures of field-grown plants during the winter (Neal, 2004). Furthermore, the moderated root-zone temperatures in PIP systems increase growth compared to AGC-grown plants (Ruter, 1998b), which will be discussed in greater detail later.

One problem associated with PIP production is the growth of the tree or shrub root system through the drainage holes of the socket pot and into the surrounding soil. This rooting-out may anchor the plant into the ground and make it difficult to harvest. Much research has been performed examining various methods, including chemical and mechanical treatments, for reducing or eliminating this problem (Ruter, 1994). Chemical treatments of Biobarrier™, Root Control™ fabric, and Spin Out™, and manually rotating the liner pot every three weeks reduced rooting out compared to control treatments (Ruter, 1994).

*Temperature*

As production of container material increases, one of the most important factors growers face is root hardiness during both winter and summer (Mathers, 2003). Nurseries wishing to expand their selection of container material may be limited by their local climate. Hardiness levels of various cultivars are common knowledge among most nursery growers, and are generally based on susceptibility of shoots to injury; however, root death may actually be responsible for most mortality of woody species (Mathers, 2003).

Healthy root systems are vital for plant growth and quality. Summer root-zone temperatures of AGC-grown plants are typically higher than ambient air temperatures due to the high heat-absorbance of the black plastic containers (Ruter, 1999a); temperatures higher than 40 °C are known to cause severe injury or death of root systems

(Mathers, 2003). Roots in the south and west quadrants of the containers, where irradiance is most intense, are most prone to injury and death (Mathers, 2003; Young and Bachman, 1996); this results in a weakened, non-uniform root ball. Since roots are more susceptible to extreme temperature fluctuations than above-ground portions of plants (Young and Bachman, 1996), one of the greatest benefits of PIP production is the moderation of root-zone temperatures, resulting in improved root health and quality (Mathers, 2003). In the southern US, where temperatures may exceed 30 °C for as many as 150 days each year (Cathey, 1998), PIP production systems protect plant root zones from the extreme heat.

Although high temperature root-kill is perceived to be a southern problem, summer root-zone temperatures in northern climates can also exceed the lethal range and cause root death in above-ground containers, particularly in the south and west quadrants of the containers (Neal, 2004). Furthermore, winter root-zone temperatures in northern climates can be lethal for plant root systems in AGC production, while PIP root-zones typically remain above the killing point. In a study in North Carolina, media temperatures of above-ground containers ranged from -2.8 °C to more than 45 °C, while root-zone temperatures in PIP production systems remained below 40 °C during the summer and above 0 °C during the winter (Hight and Bilderback, 1994). In Ohio, Zhu et al. (2005) noted that substrate temperature in the PIP system ranged between 11 and 26 °C in September compared to ambient air temperatures of 5 to 29 °C. In February, substrate temperatures were between -4 and 1 °C while the ambient air temperatures ranged between -20 and 16 °C. However, actual killing temperatures for root systems vary greatly depending on species, acclimation period, and duration of freezing temperatures.

One important consideration for PIP production is post-harvest handling. After the plant is removed from the socket pot, the surface of the liner pot is exposed to ambient conditions, such as direct solar radiation, which may result in a high heat load on the root system. The optimal root-zone conditions during production in PIP systems have been found to decrease the tolerance of plants to high temperatures during postproduction handling (Ruter, 1996). Care should be taken to provide adequate shade and irrigation during the postproduction process; some growers have also found that wrapping the containers in white plastic after harvest helps reduce the stress on the plants (Ruter, 1997).

*Niche Markets: Table-top and Living Christmas Trees*

Sales of real Christmas trees have declined over the years as artificial trees have increased in popularity (Bates, 2007; Behe et al., 2005). Niche market production of table-top and living Christmas trees is seen as one method of reviving the real tree industry (Bates, 2007). Pot-in-pot production is an ideal method for producing the container-grown trees, which can even be brought inside in time to flush for the holiday season (Genovese, 2007).

Research has shown an increasing trend toward living Christmas trees as alternatives for cut or artificial trees (Behe et al., 2005). Container-grown trees are lightweight, have excellent needle retention, can be planted and enjoyed after the holidays, and are ideal for smaller spaces (Nzokou et al., 2007). As Genovese (2007) points out, "the pot-in-pot Christmas tree successfully addresses the issue of weight, handling, survivability, monetary value, and environmental stewardship."

Display and post-handling recommendations for living Christmas trees vary. In a study by MSU researchers, pot-in-pot trees were held indoors for 20 days and then transferred to an unheated barn until the end of March when they were transplanted in the field; the trees were evaluated for three months after planting and had a 100% survival rate, although Black Hills spruce (*Picea glauca* var. *densata*) experienced extensive needle loss (Nzokou et al., 2007). Other studies suggest limiting display time to two weeks and immediately planting the trees outside in January, without an acclimation process (Bates, 2007).

**Cultural Factors**

*Fertilization: General Considerations*

In container production, plants are usually grown in soilless media, often containing pine bark and peat moss (Chong and Lumis, 2000). This can affect the cation exchange capacity (CEC), water holding capacity, and nutrient availability. Since naturally occurring nutrients may not be available for the plants to absorb, fertilization is necessary to supply micronutrients as well as macronutrients (Cregg, 2003). Growth and productivity of the majority of cultivated crops (including nursery-produced trees and shrubs) are most influenced by the addition of N-containing fertilizer (Cabrera, 2003). The fertilizer regime a nursery utilizes must be tailored to fit the production goals, whether they are to increase the size of the trees, maintain an aesthetic level, or improve plant vitality; different goals require different fertility levels (Struve, 2002).

In a review article on tree N fertilization research, Struve (2002) found that past research often lacked proper methods or rationale. Some studies were confounded by 'shared root systems', where one treatment overlapped into the root-zone of a separate treatment; other studies involved fertilizer applications during the dormant season and did not take into account species differences. The findings of the different studies resulted in highly variable N-fertilizer recommended rates, often calling for excessive amounts of N (Struve, 2002).

Conventional recommendations advise growers to apply 3 g N per gallon container (equivalent to 5.33 $g \cdot L^{-1}$ of 15-9-12 fertilizer). Following this guideline, applying even the medium or high manufacturer recommended rate of fertilizer may be insufficient for some taxa. For example, a high recommended rate of 400 g of Osmocote® Plus 15-9-12 fertilizer per 95-L (25 gal) container results in only 4.22 $g \cdot L^{-1}$.

The three main methods of fertilization used in container production are fertigation, injecting liquid-soluble fertilizer through the irrigation lines; incorporation, mixing a controlled-release fertilizer (CRF) in with the media; or top-dressing, applying CRF on top of the media (Dumroese et al., 1995). Some growers also top-dress at the beginning of the season and supplement with fertigation as the plants get larger and have greater nutrition requirements.

Nutrient uptake by plants depends on the type of fertilizer, but is also affected by temperature, media, container size, species grown, and quality of irrigation water (Struve, 2002). Ferrini and Baietto (2006) found that nutrient uptake was affected by the time of season, with maximum uptake between budbreak and fall color change. Temperature also affects fertilizer release rate, and therefore nutrient availability for uptake (Mathers, 2003; Ruter, 1998a); the moderated substrate temperatures in PIP production may slow the release-rate of CRFs and increase the longevity of the fertilizer (Ruter, 1998a).

Controlled-release fertilizers are designed to release nutrients gradually throughout the growing season, with peak release mid-season when plants are requiring the most resources (Colangelo and Brand, 2001). Fertilizers that release too early or too late in the season increase the potential for excessive nitrate leaching and wasted material. Neal (2004) found that PIP systems alter the growing patterns of trees compared to field production; the trees grown in containers grew much quicker during the first season than field-grown trees; however, during the second season, field-grown trees outgrew the container trees and differences were no longer significant. A better understanding of the growth patterns of plants grown in PIP production will enable nurseries to better time their nutrient applications.

*Fertilization: Deciduous Shade Trees*

Deciduous shade trees are typically sold according to caliper size (ANLA, 2004), and an increase of 1.25 cm (0.5 inch) in caliper can result in an additional 20 dollars or more of profit per tree for a nursery. A current concern among many growers is rapid production of shade trees for use as ash-alternatives (Cregg and Schutzki, 2004). Large amounts of fertilizer are often applied in an effort to speed caliper growth and shorten the production cycle. In a study on various species of container-grown shade tree, Murray et al. (1997) found that growth was greatest for trees fertilized with slow-release fertilizer compared to liquid or a combination of liquid and slow-release fertilizers; foliar N content, however, was lowest for trees with slow-release fertilizer. So the overall production goal, whether it is rapid growth or appearance, must be considered when selecting fertilizer methods.

Appearance is a large factor in consumer selection of landscape trees. If the goal is to provide shade, the number and size of leaves per tree would be an important consideration. Leaf color can also affect the marketability of deciduous shade trees. Fertilizer addition had a positive impact on shoot growth, leaf area, chlorophyll content, and leaf gas exchange in an urban setting (Ferrini et al., 2005).

*Fertilization: Conifers*

Nursery sales of landscape conifers are similar to shade tree sales in that appearance, such as shape and color, is an important aspect in consumer selection. In a study on Fraser fir (*Abies fraseri*), poor foliage color was related to absolute levels of nutrients as well as nutrient imbalances (Rothstein and Lisuzzo, 2006). Unlike shade-tree production, conifers are typically sold according to height, rather than caliper (ANLA, 2004), which may affect the combination of cultural factors necessary to optimize growth. In a study by Elliot and Vose (1994), availability of N affected growth of *Pinus strobus* through photosynthetic efficiency and leaf initiation and expansion. Color ratings were also correlated to height and basal diameter for *A. fraseri* throughout Michigan (Rothstein and Lisuzzo, 2006). In addition, Dumroese et al. (2005) found that transplant success of seedlings is largely based on proper nutrient regimes during production. Sometimes effects of fertilization may be difficult to quantify, but still confer additional advantages to plants. For example, proper fertilization is thought to improve cold-hardiness (Mathers, 2003).

*Media Selection*

Choosing a potting media with the proper characteristics is vital for PIP production; because container media has such a large effect on plant growth and quality, growers should not make decisions based solely on cost (Landis, 1990). Irrigation management, fertility management, weed control, and freight costs are also affected by the physical and chemical properties of container substrate. Chemical and physical properties include cation exchange capacity (CEC), total porosity, aeration porosity water holding capacity (WHC), pH, bulk density, and base fertility.

Mathers et al. (2007) state that root function, growth and morphology are greatly affected by the media properties, and aeration porosity is the most important of the factors; a substrate with low aeration porosity reduces root growth and plant development. Proper aeration porosity is essential for adequate gas-exchange of the root system, which affects water and nutrient uptake by the plant (Landis, 1990). Chong and Lumis (2000) also noted that when soil, which generally has low aeration porosity, was added to container substrate, growth of PIP trees was reduced compared to trees grown in soilless substrate. Recommendations for porosity vary greatly, however, total porosity of 50% or greater and aeration porosity of 25% to 30% is often suggested (Landis, 1990).

**Growth Responses**

*Height, Caliper, and Volume*

Many studies, encompassing a wide variety of species, have compared growth of plant material produced in PIP systems to field- or AGC-grown plants. Although results varied, the general trend was increased growth with PIP systems. Height, caliper, and biomass were greater for landscape trees grown in PIP systems compared with AGC systems (Ruter, 1998b). For southern magnolia (*Magnolia grandiflora*), PIP production increased root dry weight, combined root and shoot dry weight, height, and stem diameter compared to AGC production (Ruter, 1995). In a previous study on three landscape plants, Ruter (1993) observed that most differences between PIP and AGC production were related to improved root growth in PIP-grown plants. Zhu et al. (2005) also found that roots grow more uniformly within PIP containers due to the lack of direct heat or sun exposure compared to AGC systems.

Ruter (1998a) reported that the combination of slower nutrient release rates (a result of cooler substrate temperatures) and a larger root system often associated with PIP production increased plant growth in PIP systems since there was more fine root surface area to take up a greater amount of nutrients. The slower release rate would allow nutrients to be available over a longer period of time rather than in a single flush, potentially reducing the amount leached out and therefore unavailable for uptake. Increases in fertilizer resulted in increased canopy density and PIP trees with greater biomass than AGC trees (Ruter, 1998a). For many cultivars of landscape shade trees, doubling the amount of fertilizer applied significantly increased the total leaf area per tree (Fulcher et al., 2004), and growth is well-correlated to intercepted radiation, which is greatly influenced by leaf area (Will et al., 2001).

In a trial in New Hampshire comparing PIP production to field production, growth was not enhanced by using PIP production; however, advantages may have been conferred in other ways, such as improved root health and viability (Neal, 2004). Height, caliper, and total shoot growth of PIP trees grew more rapidly in the first season compared to field grown trees; however, during the second season the field grown trees grew rapidly, and differences between production systems were no longer significant (Neal, 2004). The container production manager at Wiegand's Nursery in Macomb, MI noticed that PIP plants flushed approximately two weeks later in the spring than AGC-grown plants because the temperatures in the containers are buffered by the surrounding soil and warm up more gradually than above-ground containers. The differing results between the trials in Georgia (Ruter, 1998a) and Kentucky (Fulcher et al., 2004) and the one in New Hampshire (Neal, 2004) may be contributed to the inherent differences, such as temperature and nutrient and water availability, between AGC and field production in addition to the climate of the experimental site.

*Leaf Area*

Plant growth can be expressed as a function of leaf area and growth efficiency, with growth efficiency defined as stem-wood production per unit leaf area (Chapman and Gower, 1991). Within a specific location, leaf area is thought to have a greater affect on productivity than growth efficiency (Allen et al., 2005). In a study on *Liriodendron tulipifera*, stem volume increments were closely related to leaf biomass; results of the study also showed that volume growth was linearly related to leaf area index (Madgwick and Olson, 1974). Simioni et al. (2004) found positive correlations between leaf number and branch basal diameter for *Crossopteryx febrifuga*, a savannah species.

**Physiological Responses**

*Photosynthetic Gas Exchange*

In a review of previous research, Elliot and Vose (1994) found that photosynthesis and productivity were not related even though photosynthesis is the carbon-forming process that drives growth. In contrast, when net photosynthesis rates of loblolly pine (*Pinus taeda* L.) were combined with leaf area data, they were largely correlated with growth (Teskey et al., 1987). This discrepancy may be caused by increased transpiration and decreased leaf conductance associated with greater tree leaf area. Ferrini et al. (2005) also found water stress effects in *Quercus robur* L. during the first year after transplanting; however, by the third year, growth and photosynthesis rates increased in response to fertilization, compared to control treatments.

*Foliar Nutrient Analyses*

Fertilization regimes are often based on visual ratings (Parent et al., 2005) which can miss symptoms of 'hidden hunger' or 'luxury consumption' (Landis and van Steenis, 2004). Foliar nutrient analyses are a common and more accurate method of monitoring the health of nursery crops (Rothstein and Lisuzzo, 2006); these are often taken in conjunction with soil samples to identify nutrient problems. Results of foliar analyses can be used to determine absolute nutrient levels or to look at ratios of one nutrient to another (Rothstein and Lisuzzo, 2006), which can help identify negative nutrient interactions. Nutrient analyses involve collecting foliar samples and sending them to a laboratory, which can be costly and time-consuming.

According to Landis and van Steenis (2003), adequate concentrations of N in plant tissues range between 1.3% and 3.5%. Literature is lacking on critical foliar nutrient levels for many landscape plants, and accepted values vary between species and regions (Landis, 1989). Some studies have determined critical nutrient levels for various conifer species, particularly those desirable as Christmas trees. In New York, Slesak and Briggs (2007) found that critical N levels for various *Abies* species and *Pseudotsuga menziesii* ranged between 1.45% and 1.75%. Rothstein and Lisuzzo (2006) recommend 1.5% as the critical N level for *A. fraseri*.

Testing for nutrient balances may be more informative and effective in diagnosing nutrient deficiencies than relying on absolute nutrient levels (Rothstein and Lisuzzo, 2006). The addition of particular elements will only improve growth and quality if other necessary elements are not limited (Slesak and Briggs, 2007).

*Indirect Measurements: Chlorophyll Fluorescence*

Indirect methods of measuring leaf chlorophyll content or concentration, such as chlorophyll fluorescence (CF) and SPAD meters, have been increasingly explored as alternatives to soil testing and plant tissue analyses (Daughtry et al., 2000). Chlorophyll fluorescence measures the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$), which is an indication of the health and efficiency of photosystem II (Krause and Weis, 1991; Groninger et al., 1996). As technology has improved, CF measuring devices have become less expensive and more portable; furthermore, they provide a "rapid, nondestructive, and objective" means of assessing CF (Ritchie, 2006). Although chlorophyll concentration may be affected by irradiance, temperature, water, and nutrients, it shows potential as indicators of nutrient deficiency (Oren et al., 1993).

If growers develop a consistent sampling regime and keep track of the values for the seasons, microclimates, and plant species, they can begin to distinguish acceptable values from low values. Ritchie (2006) found that measurements of $F_v/F_m$ were robust and only changed significantly in response to severe stress, which would be beneficial in distinguishing between temporary decreases in function, such as minor water stress reactions, and actual reduced plant health or quality.

*Indirect Measurements: Relative Chlorophyll Content (SPAD)*

According to the manufacturer of the Minolta SPAD-502 (Apogee Instruments, Inc., 2008), SPAD stands for Special Products Analysis Division, and values obtained using a SPAD meter are unitless. The meter measures the transmittance of a leaf in two wavebands, with one peak around 650 nm (red) and the other peak at approximately 940 nm (infrared). Much research has been performed in an effort to correlate the SPAD values with foliar chlorophyll or mineral nutrient concentrations (Loh et al., 2002; Parent et al., 2005). Concentrations of N and P in needles of *Abies balsamea* and *A. fraseri* were highly correlated to SPAD-502 readings (Parent et al., 2005). Since photosynthesis rate is thought to be strongly correlated with foliar N levels in many plants (Elliot and Vose, 1994), a device that measures N content could be used as a surrogate for gas exchange, thus reducing the time and effort required to monitor tree health in nursery production. Chlorophyll concentration in conifer needles is affected by age and season as well as irradiance, temperature, water availability, and nutrient supply (Oren et al., 1993). Chlorophyll and protein production in shoots are also reduced by high root-zone temperatures (Mathers, 2000). Older needles generally have a greater concentration than younger needles, and chlorophyll concentration is at its peak during summer, while carotenoids are more prevalent in winter (Oren et al., 1993).

Oren et al. (1993) points out that it is necessary for nurseries to set up a regular sampling regime in order to account for all chlorosis-causing factors and natural variations between species before chlorophyll concentration can be used as an indicator of plant nutrient deficiency. Furthermore, guidelines developed in one region should not be used to diagnose trees in another region because environmental or provenance differences may skew the results (Oren et al., 1993; Parent et al., 2005). Furthermore, Altland (2006) found that SPAD readings did not correspond to plant quality ratings for *Acer rubrum* due to the erratic readings caused by interveinal chlorosis, which was a result of Mn deficiency.

## Irrigation and Leaching

Regulations regarding irrigation use and leachate levels are becoming more stringent and nursery growers must adopt new strategies in order to comply with the requirements (Fare et al., 1996). In a study in Ohio, Zhu et al. (2005) found that most nurseries apply more water than the plants can use, which often results in leaching and runoff, both of which have potentially negative environmental impacts (Broschat, 1995; Rothstein, 2005). Since overhead irrigation is very inefficient, particularly for large containers, it is more desirable to use directed methods, such as micro-sprinklers to increase irrigation efficiency (Mathers et al., 2005). Cyclic irrigation is also shown to decrease leaching and runoff (Fare et al., 1996). With cyclic irrigation, N-leachate concentration may increase, however total N leached will decrease due to the smaller volume of water leached compared to standard single-pulse irrigation (Fain et al., 1998). Mathers et al. (2005) also noted that cultural practices that reduce root injury and heat load on the container, as in the case of PIP production, will reduce water use. When less water is applied, the potential for leaching is reduced. The amount of precipitation received should also be considered since it can be a significant source of water input and therefore increase the potential for nutrient leaching (Colangelo and Brand, 2001).

Due to its anionic nature, nitrate ($NO_3^-$) is easily leached from container substrate (Mathers et al., 2007). The U.S. Environmental Protection Agency (EPA) standard for drinking water limits the amount of nitrates present to 10 mg/L (USEPA, 2006).There are currently no regulations concerning mineral nutrient concentrations in nursery runoff; however, nurseries are now required to monitor and report on water use and leachate volumes, so it is only a matter of time before limits are established.

According to Bilderback (2001), nitrate-N levels in leachate solution should range between 50 to 100 mg $L^{-1}$ during periods of active growth in order to supply enough nutrients. Studies on leaching from field and container production have found nitrate levels ranging from 0 to 150 mg $L^{-1}$ depending on species and method of fertilization (Colangelo and Brand, 2001; Cregg et al., 2004; Wright, 1986).

Applications of slow-release fertilizer resulted in less salt concentration in leachate compared to liquid fertilizer or a combination of slow-release and liquid fertilizer (Murray et al., 1997). Combining CRF with soluble fertilizer increased nitrate leaching compared to only CRF fertilization (Yeager and Cashion, 1993). Using fertigation often results in large amounts, 32% to 60%, of N leached from the containers (Dumroese et al., 1995).

The Virginia Tech extraction method (VTEM), also called the PourThru extraction procedure, is an easy and convenient method that growers can use to monitor the pH and EC levels in the leachate from their plants (Mathers et al., 2007; Wright, 1986). Optimal pH levels vary for species, however maximum nutrient availability is around pH of 5.5 for organic substrates (Landis, 1990). One of the disadvantages of peat- and bark-based substrates is their low buffering capabilities (Mathers et al., 2007). Zhu et al. (2005) found pH levels typically ranged between 6 and 8 in leachate from a PIP production system in Ohio. Mathers et al. (2007) state that electrical conductivity values for container plants fertilized with CRFs should range between 0.2 and 1.00 dS $m^{-1}$. Regular monitoring of EC and pH levels in leachate may be used to develop fertilization regimes, with high leachate levels indicating excessive nutrient additions and wasted fertilizer.

## Literature Cited

Adrian, J.L., C.C. Montgomery, B.K. Behe, P.A. Duffy, and K.M. Tilt. 1998. Cost comparisons for infield, above ground container and pot-in-pot production systems. J. Environ. Hort. 16(2):65-68.

Allen, H.L., T.R. Fox, and R.G. Campbell. 2005. What is ahead for intensive pine plantation silviculture in the South? South. J. Appl. For. 29(2):62-69.

Altland, J. 2006. Foliar chlorosis in field-grown red maples. HortScience. 41(5):1347-1350.

ANLA. 2004. American Standard for Nursery Stock. American Nursery and Landscape Association. ANSI Z60.1-2004. 129 pp.

Anslow, D. 2007. Pot-in-pot growing and block handling. Digger. 51(11):32-37.

Apogee Instruments, Inc. 2008. 27 March 2008. http://www.apogeeinstruments.com/CCM_techinfoSPAD.htm.

Bates, R.M. 2007. Handling containerized conifers: research update. Great Lakes Christmas Tree J. 2(2):4-9.

Behe, B.K., R.M. Walden, M.W. Duck, B.M. Cregg, and K.M. Kelley. 2005. Consumer preferences for tabletop Christmas trees. HortScience. 40(2):409-412.

Bilderback, T.E. 2001. Using the PourThru procedure for checking EC and pH for nursery crops. NC State University Horticulture Information Leaflet 450.

Brooker, J.R., D. Eastwood, C. Hall, K. Morris, A. Hodges, and J. Haydu. 2005. Trade Flows and Marketing Practices within the United States nursery industry: 2003. South. Coop. Bul. 404.

Brooker, J.R., R.A. Hinson, and S.C. Turner. 2000. Trade Flows and Marketing Practices within the United States nursery industry: 1998. South. Coop. Bul. 397.

Broschat, T.K. 1995. Nitrate, phosphate, and potassium leaching from container-grown plants fertilized by several methods. HortScience. 30(1):74-77.

Cabrera, R.I. 2003. Nitrogen balance for two container-grown woody ornamental plants. Scientia Horticulturae. 97:297-308.

Cathey, H.M. 1998. The AHS heat zone map. American Horticultural Society. 25 Feb 2008. http://www.ahs.org/pdfs/05_heat_map.pdf.

Chapman, J.W. and S.T. Gower. 1991. Aboveground production and canopy dynamics in sugar maple and red oak trees in southwestern Wisconsin. Can. J. For. Res. 21:1533-1543.

Chong, C. and G.P. Lumis. 2000. Mixtures of paper mill sludge, wood chips, bark, and peat in substrates for pot-in-pot shade tree production. Can. J. Plant Sci. 80(3):669-675.

Colangelo, D.J. and M.H. Brand. 2001. Nitrate leaching beneath a containerized nursery crop receiving trickle or overhead irrigation. J. Environ. Qual. 30:1564-1574.

Cregg, B.M. and R.E. Schutzki. 2004. Recommended alternatives to ash trees for Michigan's lower peninsula. MSU extension bulletin E-2925.

Cregg, B.M., M.W. Duck, C.M. Rios, D.B. Rowe, and M.R. Koelling. 2004. Chlorophyll fluorescence and needle chlorophyll concentration of fir (*Abies* sp.) seedlings in response to pH. HortScience. 39(5):1121-1125.

Cregg, B.M. 2003. ABC's of tree nutrition: Part 2. The Landsculptor. September 2003. p. 21-23.

Daughtry, C.S.T., C.L. Walthall, M.S. Kim, E.B. de Colstoun, and J.E. McMurtre. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sensing Environ. 74:229-239.

Dumroese, R.K., D.S. Page-Dumroese, K.F. Salifu, and D.F. Jacobs. 2005. Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. Can. J. of For. Res. 35(12):2961-2967.

Dumroese, R.K., D.L. Wenny, and D.S. Page-Dumroese. 1995. Nursery waste water. The problem and possible remedies. *In* National Proceedings, Forest and Conservation Nursery Association. *Technical Coordinators* T.D. Landis and B. Cregg. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-365. pp. 89-97.

Elliot, K.J. and J.M. Vose. 1994. Photosynthesis, water relations, and growth of planted *Pinus strobus* L. on burned sites in the southern Appalachians. Tree Phys. 14:439-454.

Fain, G.B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1998. Effects of cyclic micro-irrigation and substrate in pot-in-pot production. J. Environ. Hort. 16:215-218.

Fare, D.C., C.H. Gilliam, G.J. Keever, and R.B. Reed. 1996. Cyclic irrigation and media affect container leachate and ageratum growth. J. Environ. Hort. 14:17-21.

Ferrini, F., A. Giuntoli, F.P. Nicese, S. Pellegrini, and N. Vignozzi. 2005. Influence of fertilization and soil amendments on plant growth, leaf gas exchange and on soil characteristics, J. of Arboriculture. 31(4):182–190.

Ferrini, F. and M. Baietto. 2006. Response to fertilization of different tree species in the urban environment. Arboriculture and Urban Forestry. 32(3):93-99.

Fulcher, A., W. Dumwell, R. McNiel, D. Wolfe, and L. Murdock. 2004. Effect of fertilizer rate on growth of seven tree species in pot-in-pot production. Univ. of Kentucky Agricultural Experiment Station. Nursery and landscape program research report. pr502. pp. 7-8.

Genovese, F. 2007. Growing a new market for the Christmas tree industry: pot-in-pot Christmas tree production. Great Lakes Christmas Tree J. 2(2):11, 29.

Gilman, E.F. 1988. Tree root spread in relation to branch dripline and harvestable root ball. HortScience. 23(2):351-353.

Groninger, J.W., J.R. Seiler, J.A. Peterson, and R.E. Kreh. 1996. Growth and photosynthetic responses of four Virginia piedmont tree species to shade. Tree Phys. 16:773-778.

Hight, A. and T.E. Bilderback. 1994. Substrate temperatures in above and below-ground containers in a pot-in-pot system. Proc. Southern Nursery Assoc. Res. Conf. 39:113-118.

Krause, GH and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Mol Biol. 42: 313-349.

Kuhlman, E.G., L.F. Grand, and E.M. Hansen. 1989. *Phytophthora* root rot of conifers. In Forest Nursery Pests. USDA Forest Service, Agriculture Handbook No. 680, 184 pp.

Landis, T.D. 1990. Containers and growing media. In: Landis, T.D., R.W. Tinus, S.E. McDonald, and J.P. Barnett. The Container Tree Nursery Manual, Volume 2. Agric. Handbk. 674. Washington, DC: U.S. Dept. Agric., For. Serv.: 41-85.

Landis, T.D. 1989. Mineral nutrients and fertilization. In: Landis, T.D., R.W. Tinus, S.E. McDonald, and J.P. Barnett. The Container Tree Nursery Manual, Volume 4. Agric. Handbk. 674. Washington, DC: U.S. Dept. Agric., For. Serv.: 1-67.

Landis, T.D. and E. van Steenis. 2004. Macronutrients – nitrogen: part 2. For. Nurs. Notes. Winter. 8 pp.

Landis, T.D. and E. van Steenis. 2003. Macronutrients – nitrogen: part 1. For. Nurs. Notes. Summer. 5 pp.

Linderman, R.G. and E.A. Davis. 2006. Survival of *Phytophthera ramorum* compared to other species of *Phytophthora* in potting media components, compost, and soil. HortTech. 16(3):502-507.

Loh, F.C.W., J.C. Grabosky, and N.L. Bassuk. 2002. Using the SPAD 502 meter to assess chlorophyll and nitrogen content of Benjamin fig and cottonwood leaves. HortTech. 12:682-686.

Madgwick, H.A.I. and D.F. Olson. 1974. Leaf area index and volume growth in thinned stands of *Liriodendron tulipifera* L. J. Appl. Ecol. 11(2):575-579.

Mathers, H.M., S.B. Lowe, C. Scagal, D.K. Struve, and L.T. Case. 2007. Abiotic factors influencing root growth of woody nursery plants in containers. HortTech. 17(2):151-162.

Mathers, H.M., T.H. Yeager, and L.T. Case. 2005. Improving irrigation water use in container nurseries. HortTech. 15(1):8-12.

Mathers, H.M. 2003. Summary of temperature stress issues in nursery containers and current methods of protection. HortTech. 13(4):617-624.

Mathers, H.M. 2000. Pot-in-pot container culture. The Nursery Papers. 2:1-6.

Murray, C.L., G.P. Lumis, and C. Chong. 1997. Influence of cultural factors on pot-in-pot shade tree growth. American Nurseryman. 185(6):60-61.

NASS. 2007a. Nursery crops summary 2006. National Agricultural Statistics Service and United States Department of Agriculture. 18 January 2008. http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plans

NASS. 2007b. Oregon nursery and greenhouse survey 2006. National Agricultural Statistics Service and United States Department of Agriculture. 18 January 2008. http://www.nass.usda.gov/Statistics_by_State/Oregon/Publications/Horticulture/Nursery2007final.pdf

Neal, C. 2004. Production systems for small trees and shrubs in New Hampshire. University of New Hampshire Cooperative Extension. 17 December 2007. http://extension .unh.edu/agric/AGNLT/PSSTSNH.htm

Nzokou, P., N.J. Gooch, and B.M. Cregg. 2007. Survivability of containerized live Christmas trees after indoor display. Great Lakes Christmas Tree J. 2(4):4-9.

Oren, R., K.S. Werk, N. Buchmann, and R. Zimmermann. 1993. Chlorophyll-nutrient relationships identify nutritionally caused decline in *Picea abies* stands. Can J. For. Res. 23(6):1187-1195.

Parent, L.E., L. Khiari, and A. Pettigrew. 2005. Nitrogen diagnosis of Christmas tree needle greenness. Can. J. Plant Sci. 85:939-947.

Pollock, C. and H.M. Mathers. 2002. Nursery production technique becoming a growing trend. Ohio State extension bulletin. 23 January 2002.

Powell, M.A., T.E. Bilderback, and T.M. Disy. 1996. Planting techniques for trees and shrubs. North Carolina Coop. Ext. Serv. AG 508-4. 21 May 2008. http://www.bae.ncsu.edu/programs/extension/publicat/wqwm/ag508_4/

Ritchie, G.A. 2006. Chlorophyll fluorescence: what is it and what do the numbers mean? USDA Forest Service Proceedings RMRS-P-43. pp. 34-43.

Rothstein, D.E. and N.J. Lisuzzo. 2006. Optimal nutrition and diagnosis of *Abies fraseri* Christmas trees in Michigan. North. J. Applied For. 23(2):106-113.

Rothstein, D.E. 2005. Nitrogen management in a Fraser Fir (*Abies fraseri* [Pursh] Poir.) Christmas tree production plantation: effects of fertilization on tree performance and nitrogen leaching. Forest Science. 51(2):175-184.

Ruter, J.M. 1999a. Fiber pots improve survival of 'Otto Luyken' laurel. Proc. Southern Nursery Assoc. Res. Conf. 44:37-38.

Ruter, J.M. 1999b. Production system influences growth of 'Kanzan' cherry and 'Chanticleer' pear. Proc. South. Nurs. Assoc. Res. Conf. 44:50-52.

Ruter, J.M. 1998a. Fertilizer rate and pot-in-pot production increase growth of Heritage river birch. J. Environ. Hort. 16(3):135-138.

Ruter, J.M. 1998b. Pot-in-pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. J. Environ. Hort. 16:159-162.

Ruter, J.M. 1997. The practicality of pot-in-pot. American Nurseryman. 185(1):32-37.

Ruter, J.M. 1996. Biobarrier rate influences rooting-out of five tree species produced pot-in-pot. Proc. Southern Nursery Assoc. Res. Conf. 41:104-106.

Ruter, J.M. 1995. Production system and copper hydroxide influences on growth and photosynthesis of *Magnolia grandiflora* 'St. Mary'. HortScience. 30:795.

Ruter, J.M. 1994. Evaluation of control strategies for preventing rooting-out problems in pot-in-pot production systems. J. Environmental Horticulture. 12:51-55.

Ruter, J.M. 1993. Growth and landscape performance of three landscape plants produced in conventional and pot-in-pot production systems. J. Environ. Hort. 11(3):124-27.

Simioni, G., J. Gignoux, X. Le Roux, R. Appé, and D. Benest. 2004. Spatial and temporal variations in leaf area index, specific leaf area and leaf nitrogen of two co-occurring savanna tree species. Tree Phys. 24:205-216.

Slesak, R.A. and R.D. Briggs. 2007. Christmas tree response to N fertilization and the development of critical foliar N levels in New York. North. J. Appl. For. 24(3):209-217.

Struve, D.K. 2002. A review of shade tree nitrogen fertilization research in the United States. J. of Arboriculture. 28(6):252-263.

Teskey, R.O., B.C. Bongarten, B.M. Cregg, P.M. Dougherty, and T.C. Hennessey. 1987. Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). Tree Phys. 3:41-61.

USEPA. 2006. Ground water and drinking water, consumer factsheet on: nitrates/nitrites. U.S. Environmental Protection Agency. 7 April 2008. <http://www.epa.gov/ogwdw/dwh/c-ioc/nitrates.html>.

Will, R.E., G.A. Barron, E.C. Burkes, B. Shiver, and R.O. Teskey. 2001. Relationship between intercepted radiation, net photosynthesis, respiration, and stem volume growth of *Pinus taeda* and *Pinus elliottii* stands of different densities. For. Ecol. Manage. 154:155–163.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience. 21:227-229.

Yeager, T.H. and G. Cashion. 1993. Controlled-release fertilizers affect nitrate nitrogen runoff from container plants. HortTech. 3(2):174-177.

Young, R.E. and G.R. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. J. Environ. Hort. 14:170-176.

Zhu, H., R.H. Zondag, C.R. Krause, R.C. Derksen, and T. Demaline. 2005. Preliminary investigation of water and nutrient use, substrate temperature, and moisture in pot-in-pot production. Ohio State Extension Circular 195. pp. 135-144.

# CHAPTER TWO

## FERTILIZER EFFECTS ON GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES GROWN IN POT-IN-POT SYSTEMS FOR NORTHERN CLIMATES

**Abstract**

Throughout much of the US, sales of container-produced deciduous shade trees are increasing relative to sales of balled and burlapped (B&B) material; pot-in-pot (PIP) production is an increasingly popular form of container production. In this study, we examined growth and physiological response to fertilizer addition of deciduous shade trees grown in a PIP system in Michigan. In May 2006, we potted 1- 1¼" bare root liners of *Acer* × *freemanii* 'Jeffersred', *A. rubrum* 'Franksred', *Liriodendron tulipifera*, *Platanus* × *acerifolia* 'Bloodgood', *Quercus rubra*, *Ulmus japonica* × *wilsoniana* 'Morton', and *U.* Triumph™ in 95-L containers. Treatments consisted of controlled-release fertilizer top-dressed at rates of 1.05, 2.10, 3.16, and 4.21 $g{\cdot}L^{-1}$ for the 2006 growing season, and 1.32, 2.63, 3.95, and 5.26 $g{\cdot}L^{-1}$ for the 2007 growing season. Tree growth increased with each fertilizer increment 2006. In 2007 maximum tree growth occurred at around 3.95 $g{\cdot}L^{-1}$. Total leaf area production in relation to fertilizer addition paralleled growth responses to fertilization. This supports the hypothesis that fertilizer addition increases growth by increasing total leaf area per tree. Photosynthetic response to treatment varied by species; most species had lowest rates with the 3.95 $g{\cdot}L^{-1}$ treatment, possibly a result of increased water stress associated with greater total leaf area. Chlorophyll fluorescence was not related to treatment level or foliar N concentrations; SPAD values were correlated with foliar N concentration and may be useful in detecting nutrient deficiencies, however the results were species dependent.

## Introduction

In the upper midwestern United States, including Illinois, Michigan, Ohio and Pennsylvania, gross sales of container-grown deciduous shade trees increased by nearly 95% from 2003 to 2006 (NASS, 2007a). Gross sales of balled and burlapped (B&B) material decreased by 13.5% during that time. In Oregon, often considered a bellwether for the nursery industry, container plant material outsells B&B by nearly 25% (NASS, 2007b). This shift toward containers is a response to consumer preferences (Halcomb and Fare, 1995).

Pot-in-pot (PIP) production is a large part of the overall container trend. The advantages of PIP production have become generally accepted since its introduction in the late 1980s. The system decreases windthrow, reducing the labor of righting pots, and extends the harvesting period since saturated or frozen ground is not a problem (Mathers, 2000). The system also makes it easier to control inputs, resulting in fewer potential environmental impacts due to inefficient irrigation or nitrate leaching (Ruter, 1998b) than in field and above-ground container production. Even with a relatively high startup cost, PIP production can be less expensive than conventional above-ground container (AGC) or field production due to reduced overwintering and labor costs (Ruter, 1997; Adrian et al., 1998).

Studies have shown that PIP production may increase the growth of nursery trees over field or AGC produced trees (Roberts, 1993; Ruter 1998a). This is likely a result of protection of roots from extreme temperature fluctuations that often damage roots in AGC systems (Roberts, 1993; Young and Bachman, 1996); additionally, plants grown in PIP experience less root loss at harvest compared to field-grown trees (Neal, 2004;

Mathers, 2000). One of the benefits of PIP is protection against the high summer temperatures experienced in the southern states; however, the system has also been successful in moderating root-zone temperatures during cold winter months in northern climates. A study in New Hampshire showed that roots of PIP plants experienced similar temperature ranges as field-grown plants and did not require the additional winter protection usually needed for AGC plants (Neal, 2004). For trees that are difficult to transplant, PIP could also be a method for reducing or eliminating transplant stress due to a disturbed root system. Although a study in Georgia showed species dependent effects on field performance of landscape plants grown in AGC and PIP systems (Ruter, 1993), other researchers have found clear benefits of PIP compared with field-grown plants (Nzokou et al., 2007).

What has been lacking so far is research on the physiological response to trees in the PIP system. Nitrogen is essential for making amino acids, enzymes and chlorophyll, and consequently leaf chlorophyll concentration has been shown to correlate to plant N content (Daughtry et al., 2000). However, deficiency is expressed differently in different species; likewise, species respond differently to nutrient availability. Some species increase the number of leaves per tree while others increase the average specific leaf area (Haase and Rose, 1995). It is possible to increase growth by increasing either total leaf area or growth efficiency (growth per unit leaf area).

Understanding which factors are affected by cultural practices and which are species dependent will aid growers in cultivar selection as well as designing management practices. Once the trees are selected and in production, the next step is to continually monitor their health so as to maintain optimum productivity. This can be difficult and time consuming if many samples have to be collected and sent to a lab for analysis. A desirable alternative is to correlate simple test results with overall tree health. Chlorophyll fluorescence measures the photosynthetic efficiency by calculating the ratio of variable fluorescence to maximal fluorescence ($F_v/F_m$). Low $F_v/F_m$ values often correspond to plant stress (Ritchie, 2006), and have been also related to high soil pH (Cregg et al., 2004). Since pH can affect uptake of many nutrients, including B, P, Zn, and Cu, $F_v/F_m$ is likely a non-specific indicator of nutrient deficiency (Cregg et al., 2004). Minolta SPAD 502 is another portable tool that has been used to estimate chlorophyll levels by measuring transmittance of two wavelengths of light through leaves. SPAD meter readings were highly correlated to chlorophyll content of fig and cottonwood leaves, but less related to N content (Loh et al., 2002). Fritschi and Ray (2007) did find a correlation between N content and SPAD readings for soybeans, however the relationship was not strong enough to eliminate the need for foliar analysis. Similar to fluorescence values, SPAD values can be confounded by other factors such as water stress and seasonal variations (Davenport et al., 2005).

Many studies have compared PIP production with field or conventional AGC production, but growers still lack recommendations for selecting cultural factors specific to PIP production, especially in northern climates. The present study was carried out as part of a program to optimize growth and efficiency within the PIP production system for the upper Midwest region. The overall goal of the study was to formulate guidelines for PIP production to maximize productivity and minimize cost and negative environmental impacts. We hypothesized that tree growth was dependent on total leaf area (TLA; i.e. the ability of the tree to photosynthesize), and therefore, increasing TLA would result in increased growth. Specific objectives were to 1) determine the growth and physiological responses to varying nutrition rates, 2) minimize environmental impact and grower expense by identifying target nutrition rates, 4) evaluate nutrient diagnostics using indirect methods such as SPAD or chlorophyll fluorescence, and 5) use the resulting diagnostics to develop foliar nutrient concentration guidelines to allow growers to monitor the health of their trees.

## Materials and Methods

### *Site Description*

This experiment was conducted at the Michigan State University (MSU) Horticulture Teaching and Research Center, Lansing, MI. The soil was loamy sand (83.1% sand, 8.7% silt, 9.3% clay), which provided adequate drainage of the containers in the PIP system. We used a 90 cm diameter auger mounted on a skid-steer to drill holes in order to install socket pots; holes were spaced 1.4 m on-center within rows, and 1.8 m on-center between rows, with rows offset. Landscape cloth was laid down over the holes and secured with standard landscape staples. We cut circles from the fabric over each hole and placed 95-L (25-gal) socket pots (GL10000, Nursery Supplies, Inc., Chambersburg, PA) so the rims were approximately 2.5 cm above the surface of the ground.

The mean daily air temperature during the growing season (June through September) was 25.3 °C for 2006 and 26.4 °C for 2007; total precipitation during that time was 318 mm in 2006 and 295 mm in 2007 (MAWN, 2007). Average air temperature during the winter months (December 2006 through March 2007) was −1 °C with a minimum of −22 °C (MAWN, 2007).

*Plant Materials*

In May 2006, we potted 160 bare-root liners (20 of each species or cultivar; initial caliper of 2.5 – 3 cm) of *Acer ×freemanii* E. Murray (*rubrum* × *saccharinum*) 'Jeffersred', *A. rubrum* L. 'Franksred', *Liriodendron tulipifera* L., *Platanus acerifolia* (Aiton) Willd. 'Bloodgood', *Quercus rubra* L., *Ulmus japonica* (Rehder) Sarg. × *wilsoniana* C.K. Schneid. 'Morton', *U. pumila* L. × *japonica* (Rehder) Sarg. × *wilsoniana* C.K. Schneid. 'Morton Glossy' Triumph™ and *Celtis occidentalis* L. (from J. Frank Schmidt and Son Co., Boring, OR) into 95-L containers (EG10000, Nursery Supplies, Inc., Chambersburg, PA). Substrate was composed of 85% composted pine bark and 15% Canadian peat moss (by volume), amended with 0.9 kg sulfur and 0.45 kg ferrous sulfate per 0.76 $m^3$ with a target pH of 5.5 (Renewed Earth, Inc., Kalamazoo, MI).

*Fertilizer Treatments*

Trees were top-dressed using controlled-release fertilizer (Osmocote® Plus 15-9-12, 8-9 month Northern release rate at 21 °C, The Scotts Co., Marysville, OH) containing 7% $NH_4$-N, 8% $NO_3$-N, 3.9% P, 10% K, 1% Mg, 2.3% S, 0.02% B, 0.05% Cu, 0.45% Fe, 0.06% Mn, 0.02% Mo, 0.05% Zn in the spring of each season. Rates selected in 2006 corresponded to high (4.2 g fertilizer $L^{-1}$ container), medium (3.16 g $L^{-1}$) and low (2.11 g $L^{-1}$) recommended rates as well as one-half the low recommended rate (1.05 g $L^{-1}$) used to observe low nutrient responses. For the 2007 season, the rates were increased by 25% to accommodate increased tree nutrient requirements.

*Irrigation*

Irrigation was initially applied using an overhead pressure sprinkler. In early August 2006, two micro-sprinkler spray stakes (TS-90, Chapin Watermatics Inc., Water

Town, NY) were installed per container and set to run twice per day using an automated valve (8014 DuraLife, L.R. Nelson Corp., Peoria, IL), at 7:15 AM and 5:45 PM, for 8 min each cycle. The output of each stake was set to the medium level, supplying approximately 0.4 L $min^{-1}$, for a total of approximately 12 L of water applied daily per tree. Trees were irrigated from early May to mid-November of both years.

*Experimental Design*

The experimental design was a split-plot in a randomized complete block with five blocks. Trees of the same taxa were placed together in groupings of four trees per species to reduce the effect of large-canopy species shading species with smaller canopies. The four treatment levels were randomly assigned within each group of species, with one treatment per tree. Blocks were used to account for diurnal variations in physiological responses.

*Growth*

Initial height and caliper for the 2006 season were measured 16 May 2006 using a standard height pole and digital hand calipers. Height was measured from the rim of the pot to the tip of the leader. We measured caliper 33 cm above the rim of the pot with the calipers oriented east-west. Marks were drawn on the trunks of the trees with a permanent marker so subsequent caliper measurements were taken at the same place. Final height and caliper for the 2006 growing season were measured on 30 August. Trees were pruned at the end of the 2006 season according to standard nursery practices. Initial height and caliper for 2007 was taken on 7 May, and final growth data were collected 7 November 2007.

Total height and caliper growth for each season was calculated by subtracting the initial height or caliper from the final height or caliper. Stem volume indices for each season were calculated by multiplying the square of the caliper growth by the height growth for each season ($d^2h$). In order to account for species-related differences in initial stem caliper, which could affect absolute growth, caliper relative growth rate (RGR) was also calculated for each tree by subtracting the initial caliper from the final caliper and then dividing by initial caliper. This was done separately for each year.

*Gas Exchange*

We measured gas exchange with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) on 6 and 30 June, 31 July, and 7 September 2006. For the 2007 season, measurements were taken on 22 May, 26 June, 10 July, and 15 and 31 August. On each date, measurements were taken between 9:00 AM and 5:00 PM; data were collected from all blocks on the same day. Light saturated photosynthesis ($A_{max}$), conductance ($g_{wv}$), and transpiration (E) were measured on mature leaves exposed to full sun with a 3 × 2 cm leaf chamber with a red/blue LED light source (Li-6400-02B, Li-Cor) to maintain quantum flux at 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Gas exchange measurements were recorded after photosynthesis and conductance rates displayed on the real-time graphics system of the instrument had stabilized. Air flow into the chamber was 500 $\mu mol \cdot s^{-1}$, with a constant reference $CO_2$ concentration of 400 $\mu mol\ CO_2 \cdot s^{-1}$. In order to reduce temperature variation across a daily set of measurements, block temperature within the chamber was set to the predicted high air temperature on the day of measurement. Intrinsic water use efficiency (WUE) was calculated as WUE = $A_{max}/g_{wv}$.

*Chlorophyll fluorescence*

A portable chlorophyll fluorescence meter (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England) was used to measure the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$) on individual leaves from each tree. The plastic/foam clips provided by the manufacturer were clipped to leaves exposed to full sun, and the leaves were allowed to dark-acclimate for 15 min before readings were taken. Dates of measurement of $F_v/F_m$ coincided with measurements of $A_{max}$.

*Relative chlorophyll content*

We used a Minolta SPAD-502 meter (Spectrum Technologies, Plainfield, IL) to measure the relative chlorophyll content on individual leaves. The hand-held device was pressed against the leaf for a few seconds and the reading, a value proportional to leaf chlorophyll content, was recorded. Measurements were repeated three times per tree. Collection dates coincided with dates of $A_{max}$ measurement.

*Canopy Light Interception*

Photosynthetic photon flux (PPF) was measured under each tree canopy using a 1 m long, 10-sensor, hand-held quantum light meter (Apogee Inst., Logan, UT); open-sky measurements were also taken every four trees. Ten values, five in a north-south orientation and five in an east-west orientation, were taken approximately half-way between the trunk and the edge of the canopy for each tree, and averaged to determine the transmission of the canopy. Interception for each tree was determined by subtracting the average canopy transmission value from the full-sun value taken just prior to the measurement of the tree.

*Predawn and Midday Water Potential*

Predawn and midday stem water potentials were measured for a subsample of trees on 23 May, 7 June, 20 June, 10 July (midday only), 24 July (predawn only), and 29 August 2007. The dates correspond to dates of $A_{max}$ measurements. Trees with low (1.32 g $L^{-1}$) and high (5.26 g $L^{-1}$) fertilizer treatments were sampled for each species. For predawn measurements, leaves were collected approximately 1 hr before sunrise, sealed in zippered plastic bags, and placed in a cooler for transportation to the laboratory. Within 1 hr of collection, water potential was measured using a pressure chamber instrument (Model 600, PMS Instrument Co., Corvallis, OR). Each leaf was secured in the pressure chamber lid using a rubber stopper so the cut end of the petiole remained outside the chamber and the leaf blade was sealed inside. The chamber was pressurized with nitrogen, and the cut end of the petiole was watched carefully for the first appearance of a sap bubble; the corresponding pressure was recorded.

For midday water potential, two leaves per tree were wrapped in aluminum foil by 9:30 AM in order for the leaf water potential to come into equilibrium with the stem water potential (Bogart, 2006; Shackel et al., 1997). The foil-wrapped leaves were collected between 11:30 AM and 1:30 PM EST (encompassing solar noon), sealed in zippered plastic bags, and placed in a cooler for transportation to the laboratory. Within approximately 1 hr of collection, water potential was measured in the same way as for the predawn measurements; the two values per tree were recorded and averaged.

*Leaf area*

Foliar samples were collected 31 July 2006 and 3 September 2007; 15 leaves were collected from each tree, 5 each from the lower, middle, and upper canopy in order to obtain a full representation of sun and shade leaves. The leaves were scanned using a leaf area meter (LI-3000, Li-Cor Inc., Lincoln, NE) and the values were averaged for each tree. The 15 leaves were then placed in paper bags and oven-dried at 60 °C to a constant weight. Specific leaf area (SLA) was calculated by dividing the average area of the 15 leaves by the mass of the sample. Leaf litterfall was collected at the end of the 2006 season by netting every tree canopy with 1 or 2 cm mesh bird netting and securing it below the branches using zip-ties or duct tape. After the leaves had fallen, the nets were removed and the leaves were collected in paper bags. The leaves were oven-dried at 60 °C and weighed. Total leaf area (TLA) was calculated by multiplying the SLA by the total leaf weight for each tree. In October and November of 2007, just prior to leaf-abscission, all leaves were manually removed from each tree and stored in paper bags. Due to high winds, some trees lost their leaves before collection was possible. The leaves were oven-dried at 60 °C and weighed to determine TLA for 2007.

*Foliar Analysis*

The leaves collected for calculation of SLA and TLA were ground using a standard coffee bean grinder until they passed through a 0.42 mm mesh sieve, and were sent to a commercial laboratory (Waters Agricultural Laboratories, Inc.,Camilla, GA) for analysis. The leaves collected in July 2006 were analyzed for full foliar nutrient content; leaves collected at the end of the 2006 season and in September 2007 were analyzed for foliar nitrogen. Percent retranslocation of N was calculated for all trees, and percent re-translocation of all nutrients was calculated for subsample of trees at the end of the 2006 growing season using the equation:

$$\%\ retranslocation = \frac{(July\ concentration) - (litterfall\ concentration)}{July\ concentration} \times 100$$

Leaves collected at the end of the 2007 season were not analyzed; since the leaves were pulled from the trees prior to natural senescence, a foliar analysis would not have reflected fully retranslocated nutrient levels.

*Nitrate, pH, and EC in leachate*

We used the PourThru extraction procedure (Bilderback, 2001) to collect leachate from a subsample of three species, *U.* Triumph™, *A. rubrum* 'Franksred', *P. × acerifolia* 'Bloodgood', representing small, medium, and large canopies, respectively. The leachate was collected approximately every 2 wk throughout the growing season using 20 mL plastic vials, which were stored in a 2.5 °C cooler until analysis. Within a week after collection, we measured pH (Accumet® Basic AB15 meter, Thermo Fisher Scientific Inc., Waltham, MA) and electrical conductivity (EC; ExStik II EC500, Omni Controls Inc., Tampa, FL) in the laboratory. Nitrate-N analysis was conducted by the MSU Soil and Plant Nutrient Laboratory (SPNL) using flow-injection with cadmium reduction (Huffman and Barbarick, 1981).

*Statistical Analysis*

Statistical analyses were performed using SAS software 9.1 (SAS Institute Inc., Cary, NC). All variables were tested for normality using PROC UNIVARIATE and Levene's test. Caliper growth and EC data were normalized using a cuberoot transformation Total height growth was normalized using a squareroot transformation. A log transformation was used to normalize height, and stem volume growth; log (x) was also used to normalize conductance, transpiration, total leaf area, and total leaf dry weight.

PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. Effects over time for gas exchange, nitrate-N, pH, EC, and $F_v/F_m$ data were analyzed using repeated measures within PROC MIXED. Pearson correlation coefficients for stem caliper, volume, TLA, $A_{max}$, E, SPAD, N, P, K, Mg, Ca, Fe, Cu, pH, and $NO_3$ were determined using PROC CORR. Pearson's correlation coefficients were determined between foliar N levels and SPAD values. Mean separation using Tukey's adjustment was used to compare SLA, total leaf dry weight, and TLA response to fertilization.

*Considerations*

All *C. occidentalis* experienced extreme transplant shock, did not flush in spring of 2006, and were removed from the study.

To control Japanese beetles, which were causing extensive defoliation, especially on the elm cultivars, the entire plot was sprayed with Sevin (Southern Agricultural Insecticides, Inc., Palmetto, FL) on 21 July 2006 at a rate of 1.8 $g \cdot L^{-1}$, and again on 9 August 2006 at a rate of 2.4 $g \cdot L^{-1}$. Guthion (Bayer, Inc., Toronto) was applied on 3 July 2007 at a rate of 2.4 $g \cdot L^{-1}$.

## Results

### *Growth Responses*

*Caliper, Height, and Volume*

Growth, in general, was greater during the second season than the first, likely reflecting the increased fertilizer treatment levels applied in 2007 compared to 2006 (fig. 1.1); in addition, the trees were larger, and therefore had more potential for growth. Furthermore, trees probably experienced some transplant shock during the first season, which was not a factor during the second season. Since the fertilizer treatments were not the same for both years, the growth parameters were analyzed separately by year to better examine the species and treatment effects.

Stem caliper relative growth rate (RGR) was affected ($p<0.0001$) by fertilizer treatment in 2006, while species was the only influence ($p<0.01$) in 2007 (table 1.1). Averaged across species and treatments, relative growth rates were over 50% greater in 2007 compared to 2006 (data not shown).

Stem caliper was influenced ($p<0.0001$) by both species and fertilizer treatment in 2006 and 2007 (table 1.1, fig. 1.1). In 2006, caliper growth increased with additional fertilizer, although there were no differences between the 2.10 and 3.16 g fertilizer per L treatments or between the 3.16 and 4.21 g treatments when averaged across species (fig. 1.1). For 2007, the 5.26 $g \cdot L^{-1}$ treatment did not increase growth compared to either the 2.63 or 3.95 g treatment. Furthermore, total caliper increase in 2007 was typically greater than total caliper increase in 2006 (fig. 1.2). Species response to fertilizer addition varied between species; some responses were linear, while others were quadratic (fig. 1.2). Stem caliper was not consistently greater or lesser for any given species over the two-year cycle. In contrast, when averaged across species, the fertilizer trends were clear for both years.

Treatment did not influence height growth during either season (table 1.1, fig. 1.1), however, species affected ($p<0.0001$) height in 2007. Stem volume growth, in comparison, was affected ($p<0.01$) by species and fertilizer treatment in both 2006 and 2007 (table 1.1). The trends for stem volume were similar to those of caliper growth.

*Leaf Area*

Specific leaf area (SLA) was largely species-dependent ($p<0.0001$) (table 1.2). The species × year interaction was also significant; however, *Liriodendron tulipifera* had the highest SLA compared to the other species for both years, and the *Ulmus* cultivars had consistently low SLA. In addition to species effects, fertilizer levels also influenced ($p<0.05$) SLA (table 1.2). When the data were averaged across species, SLA generally increased with increasing fertilizer addition (fig. 1.3).

Total leaf dry weight per tree was affected ($p<0.0001$) by fertilizer treatment and species. In 2006, the 3.16 and 4.21 $g \cdot L^{-1}$ treatments increased dry weight compared to the 1.05 treatment. Dry weight was greater for the 2.63, 3.95, and 5.26 $g \cdot L^{-1}$ treatments compared to the 1.32 treatment in 2007. The *Acer* cultivars and *P.* × *acerifolia* had high dry weights both years, while *L. tulipifera* and *U.* Triumph™ had low dry weights compared to the other species (data not shown). The species × year effect was significant ($p<0.0001$), particularly because of *Q. rubra*. Total leaf dry weight for *Q. rubra* was much greater in 2007 than in 2006. Total dry weights were not available for *U. japonica* in 2007 due to heavy insect pressure and high winds, which removed the majority of leaves before collection was possible.

For the 2006 season, TLA was higher for 2.10, 3.16, and 4.21 g fertilizer per liter of container compared to the 1.05 g treatment; furthermore, adding 3.16 and 4.21 g per L increased TLA compared to the 2.10 g treatment (fig. 1.3). In 2007, TLA was higher for 2.63, 3.95, and 5.26 $g \cdot L^{-1}$ of container compared to 1.32 g, but there were no differences in TLA between the three highest fertilizer treatments (fig. 1.3). These trends parallel those of caliper growth (fig. 1.4a) and stem volume (data not shown), supporting the hypothesis that overall tree growth was driven by total leaf area in this study. Averaged across treatments, TLA was greatest for *A. × freemanii*, *A. rubrum*, and *P. × acerifolia*. Overall greater TLA in 2007 compared to 2006 likely reflect greater overall tree size.

*Canopy Light Interception*

Canopy light interception (CLI) was only measured during the second growing season. Three sets of measurements were taken, but analysis was conducted using only the data from 23 July 2007 since the leaves were not fully expanded on the two earlier dates. Species influenced ($p<0.0001$) CLI (table 1.2); the *Ulmus* cultivars had greater interception than *P. × acerifolia* and *Q. rubra* reflecting their denser crown structure. Interception was also affected ($p<0.05$) by fertilizer addition, since the 1.32 $g \cdot L^{-1}$ treatment had a lower CLI than the other treatments. The CLI response to fertilizer addition was similar to those of TLA and stem volume growth.

***Physiological Responses***

*Photosynthetic Rates*

Photosynthetic rates were affected ($p<0.01$) by fertilizer treatment in 2007 but not in 2006 (table 1.3). Although the treatment × date interaction was also significant ($p<0.01$) in 2007, $A_{max}$ was generally higher with lower fertilizer additions (fig. 1.6). Leaf conductance to water vapor and $A_{max}$ were negatively correlated with total canopy leaf area ($p<0.0001$) (table 1.4), indicating greater water demand associated with a larger canopy. Photosynthetic rates were also species dependent ($p<0.0001$) for both years (table 1.3). Averaged across treatments, the *Ulmus* cultivars and *P. × acerifolia* typically had the highest $A_{max}$. In 2006, $A_{max}$ ranged between 4 and 16 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for most species, and the general trend was an increase in $A_{max}$ as the season progressed (fig. 1.5a and b). Average rates were slightly lower in 2007, ranging between 2 and 12 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for most species.

Fertilizer treatment also influenced ($p<0.05$) conductance (fig. 1.6). When averaged across species, conductance decreased with increased fertilizer addition for both years. Conductance was affected ($p<0.0001$) by species in both 2006 and 2007 (table 1.3). Both *Ulmus* species, as well as *P.* × *acerifolia*, had high conductance values in 2006; in 2007, most species had similar values expect for *U.* Triumph™, which was high, and *Acer* × *freemanii*, which was low.

As with the growth responses, the species × treatment interaction was not significant for any of the gas exchange responses (table 1.4).

*Water Use Efficiency*

Water use efficiency (WUE; $A_{max}/g_{wv}$) was affected by species ($p<0.0001$) in 2006, and by treatment level ($p<0.0001$) in 2007 (table 1.3). Averaged across species, water use efficiency (WUE) increased with fertilizer addition (fig. 1.6). *Quercus rubra* had lower ($p<0.05$) WUE compared to all the other species except *U.* Triumph™ (fig. 1.7).

*Leaf Water Potential*

Midday water potential ($\Psi_w$) was affected ($p<0.05$) by treatment level (table 1.5). The 5.26 $g \cdot L^{-1}$ fertilizer treatment had lower $\Psi_w$ (-13.4±0.33SE bar) than the 1.32 $g \cdot L^{-1}$ treatment (-12.6±0.30SE bar), indicating that the trees with higher fertilizer levels were under greater water stress than the trees given the low rate of fertilizer. Both predawn and midday $\Psi_w$ differed ($p<0.0001$) between species (table 1.5). When averaged across date and treatment level, the *Ulmus* species had consistently low predawn $\Psi_w$ and *A.* ×

*freemanii* and *P.* × *acerifolia* had high predawn $\Psi_w$ (fig. 1.8a). Average values for midday $\Psi_w$ were lower than for the predawn measurements (fig. 1.8b).

***Foliar Nutrient Concentrations***

*Mid-season Foliar Analyses*

Fertilizer treatment affected ($p<0.0001$) N, P, and K foliar concentrations (table 1.6); averaged across all species, concentrations increased with fertilizer addition for all three nutrients. Photosynthetic rates were negatively correlated ($p<0.0001$) with N (table 1.4). Nitrogen concentration and caliper growth were also highly related both years ($R^2 > 0.95$) (fig. 1.4b). Foliar concentrations of N, P, and K were highly significant ($p<0.0001$) for species (table 1.6). The *Acer* species and *P.* × *acerifolia* had low N levels compared to the other species for both years. *Ulmus* Triumph™ had somewhat high P and K levels compared to the other species while *L. tulipifera* had consistently low levels; *P.* × *acerifolia* and *U. japonica* also had relatively low P levels.

*Litterfall Foliar Analyses*

When the data were averaged across species, the two lower fertilizer treatments exhibited greater amounts of retranslocation (fig. 1.9b). This, however, was not true for *Quercus rubra*, which had high rates for all treatments. Percent N retranslocation for litterfall leaves compared to samples collected mid-season was affected ($p<0.0001$) by species (table 1.7). *Quercus rubra* had much greater retranslocation, conserving nearly 60% of its nutrients, compared to the other species. *Platanus* × *acerifolia* had the lowest rate, at only 20% (fig. 1.9a).

*Indirect Measurements*

Relative chlorophyll content, measured with the SPAD-502 meter, was affected ($p<0.0001$) by species and fertilizer treatment (table 1.8). Although the species × date interaction was also significant ($p<0.0001$), *A.* × *freemanii*, *Q. rubra*, and *P.* × *acerifolia* had consistently low values compared to the other species. During the first season, differences between treatments were minor with no clear trend; however, in 2007, SPAD values typically increased with increasing fertilizer addition. The species × treatment interaction was also significant ($p<0.0001$) in 2007 (table 1.8). Pearson's correlation coefficients indicated no relationship between SPAD and foliar N when the species were analyzed together (table 1.4). When the data were plotted separately by species and fit with regression curves, the values were positively related ($R^2 > 0.85$) for *A.* × *freemanii*, *L. tulipifera*, *P.* × *acerifolia*, and *Q. rubra* (fig. 1.10).

SPAD readings were correlated with $A_{max}$ rates ($p<0.0001$), indicating possible use as an alternative diagnostic tool for plant health; however, correlations between SPAD values and foliar N concentrations are species-dependent. SPAD values were negatively correlated with P and K ($p<0.0001$) (table 1.4).

For both the 2006 and 2007 seasons, chlorophyll fluorescence ($F_v/F_m$) was affected ($p<0.0001$) by species, but not by treatment (table 1.8); consequently, correlations between $F_v/F_m$ and foliar N concentrations were not significant.

***Leaching***

Across all species, $NO_3$ and EC levels in leachate increased with increasing fertilizer addition ($p<0.0001$), while leachate pH decreased with increased fertilizer ($p<0.0001$) (table 1.9). Throughout the 2006 season, leachate $NO_3$ concentration reached a maximum in mid-July for all treatments and differences between treatments were no longer different by mid-August (fig. 1.11). Average concentrations were greater for the 2007 season, consistent with the increase in fertilizer treatment levels, and reached a maximum between mid-June and mid-July. By the end of August, the differences between treatments were no longer significant. The same trends were observed for EC levels in leachate. In 2006, the EC values for the 4.21 $g \cdot L^{-1}$ treatment ranged between 1800 at the beginning of the season to 550 at the end of the season; the 1.05 $g \cdot L^{-1}$ treatment ranged between 1000 and 450. In 2007, EC levels peaked mid-season, and ranged between 2100 and 1000 for the 5.26 $g \cdot L^{-1}$ treatment and between 1050 and 650 for the 1.32 $g \cdot L^{-1}$ treatment. The average initial pH, taken 23 June 2006, was 6.12 (±0.04 SE) when averaged across all treatment levels. Throughout the study, leachate pH levels ranged between 6 and 7.5 (fig. 1.11).

**Discussion**

This study was designed to examine how fertilizer rates affect growth of deciduous shade trees in PIP production systems in Northern climates. The overall goal was to optimize tree growth and efficiency within the PIP production system. When examining the results of the study, the objectives were to: 1) determine growth and physiological responses to varying nutrition rates; 2) determine the feasibility of using indirect methods, such as SPAD meters, to monitor overall tree health; and 3) develop nutrient addition recommendations for growing deciduous shade trees in PIP production systems.

***Growth Responses***

Tree growth is a function of leaf area and growth efficiency (growth per unit leaf area) (Allen et al., 2005; Fox et al., 2007). The biomass response most relevant in this case is stem caliper, since that is the basis for profitability in nursery production. Results of the current study suggest that growth response of nursery trees to nutrition is driven largely by changes in leaf area rather than growth efficiency.

*Caliper, Height, and Volume*

Stem caliper, height, and volume growth tended to increase with increased fertilizer addition; however, applications greater than 3.95 $g \cdot L^{-1}$ container did not increase growth. This implies that the extra fertilizer was either not taken up by the trees, or possibly resulted in luxury consumption. Since the species × treatment interaction was not significant for any of the growth responses, this indicates that fertilizer recommendations apply to all of the species studied.

*Leaf Area*

Increasing total leaf area (TLA) is one means of potentially increasing growth. Total leaf area, averaged across all species, generally increased with additional fertilizer in 2006. This is consistent with previous studies which found that increased fertilizer additions were correlated with increased leaf area and greater canopy biomass (Fulcher, et al., 2004; Ruter, 1998a). In 2007, maximum TLA occurred with the 3.95 g treatment. This again indicates that addition of fertilizer beyond 4.21 $g \cdot L^{-1}$ is not necessary or even beneficial for the species examined. Since stem growth responses paralleled those of TLA, and TLA develops early in the season while stem growth continues throughout the season, this supports the hypothesis that growth is driven by increases in leaf area.

*Canopy Light Interception*

Will et al. (2001) found correlations between growth and intercepted radiation. In this study, canopy light interception values were related to fertilizer addition, and were also affected by species. Increased interception was associated with greater leaf area, which generally increased with fertilizer addition, and was often associated with increases in growth.

***Physiological Responses***

*Photosynthetic Rates*

Improved growth efficiency, in addition to an increase in leaf area, is another means by which trees could increase growth in response to fertilization. Photosynthetic rates are one of the key factors in determining growth efficiency. Throughout both seasons, $A_{max}$ was relatively unresponsive to fertilizer treatment, or decreased with increased fertilization. Some species, such as the *Ulmus* species and *P.* × *acerifolia* consistently had highest rates of $A_{max}$ associated with the lowest fertilizer treatment, particularly in 2007. The lack of a treatment effect on $A_{max}$ further suggests that growth was not increased through greater efficiency. We hypothesize that increased canopy leaf area associated with fertilization resulted in greater canopy transpiration and, consequently, increased water stress and partial stomatal closure. This supposition is supported by trends in leaf conductance, which declined with increased fertilization and were negatively correlated with TLA.

*Water Use Efficiency*

Increases in intrinsic water use efficiency (WUE; $A_{max}$ / $g_{wv}$) were associated with higher fertilizer levels, particularly in the second season. This indicates that even though $A_{max}$ did not increase with fertilization, the trees were still responding to the extra nutrients. Water use efficiency was also species-dependent ($p<0.01$); among species, *Q. rubra* had the lowest WUE. It is interesting to note that the *Ulmus* species had consistently low SLA and high WUE compared to the other species. This suggests a relationship between those two responses, but could also be confounded by Japanese beetle damage, which was prevalent on the *Ulmus* species.

*Leaf Water Potential*

The species effect on the predawn water potentials ($\Psi_w$) is likely due to differences in xylem anatomy and hydraulic architecture (Tyree, 1988). Midday water potentials were affected by treatment as well as species. The fact that water potentials ($\Psi_w$) were lower with the higher fertilizer levels is yet another indication that the larger trees were under greater water stress, which likely limited their photosynthetic potential and growth.

***Nutrient Diagnostics***

Both SPAD and $F_v/F_m$ have been proposed as indirect indicators of foliar nutrient status (Daughtry et al., 2000). In this study, relative chlorophyll content, measured using a SPAD-502 meter, was positively correlated with $A_{max}$, P, and K. Foliar N and P concentrations have also been correlated with SPAD readings for *Abies balsamea* and *A. fraseri* (Parent et al., 2005). Conversely, Altland (2006) found that SPAD readings were not useful in measuring *Acer rubrum* plant quality since Mn-induced interveinal chlorosis resulted in erratic measurements. For all the species and treatments we tested, foliar N concentrations were above the suggested critical level 1.5% N for both years, so we were unable to evaluate the usefulness of the SPAD meter in determining N deficiency. The wide range in SPAD values for different species as well as the significant species × treatment interaction indicate that results must be evaluated separately for each species.

Chlorophyll fluorescence ($F_v/F_m$) was not related to treatment level in either year, nor were $F_v/F_m$ and foliar N concentrations correlated. These results indicate this method of indirect measurement is not useful in determining plant nutrient levels for the species tested. Ritchie (2006) also found that $F_v/F_m$ was robust and only changed in response to severe stress. Furthermore, $F_v/F_m$ is associated with deficiencies of many mineral elements, so results may only be useful as a non-specific indicator of plant stress (Cregg et al., 2004).

***Nutrition Recommendations***

In nursery production of deciduous shade trees, nutrient additions are used to increase caliper and improve aesthetics, thereby improving the marketability of the trees. Increasing the amount of fertilizer per treatment from 2006 to 2007 helped distinguish the optimum range of nutrition required for maximizing tree growth. Foliar analyses were also performed to determine if nutrient uptake coincided with the amount of nutrients applied or if negative interactions were inhibiting uptake.

Recommended foliar N concentrations for nursery trees range between 1.3% and 3.5% (Landis and van Steenis, 2003). This is broad range and is inclusive of many different species. Nitrogen concentrations measured for all species and treatments in this study were greater than 1.5%, indicating that even at low fertilizer levels, N was not limiting. Although adding 5.26 $g{\cdot}L^{-1}$ did not increase growth compared to adding 4.21 $g{\cdot}L^{-1}$ concentrations of N, P, K, and Mg continued to increase suggesting luxury consumption.

Retranslocation of N between litterfall leaves and foliar samples collected mid-season was calculated using the concentrations on a mass basis. This does not take into account the loss in weight due to loss of minerals or leaf senescence, but is an acceptable estimate (Huang et al., 2006; Luyssaert et al., 2005). Results from this study support the general hypothesis that trees under nutrient stress conserve a greater percent of their resources than species with sufficient levels of available nutrients (Huang et al., 2006).

In conclusion, growth increased with the addition of fertilizer up to a point, but is ultimately constrained by leaf area development. Applying fertilizer beyond the recommended 5.33 $g \cdot L^{-1}$ (equivalent to 3 g N per 1 gal) container did not further increase growth, even though foliar mineral nutrient concentrations were greater. Correlations between SPAD values and foliar N concentrations indicate that the SPAD meter could be used to determine nutrient deficiency, but the results were species and date dependent. The apparent negative feedback between leaf area and $A_{max}$ could possibly have been overcome by better irrigation management (Fain et al., 1998), however, that was beyond the scope of the present study.

Table 2.1. Summary analysis of variance for caliper growth, caliper relative growth rate (RGR), height growth, and volume growth per year for seven taxa of deciduous shade tree grown in a PIP production system under four rates of fertilization.

| Source of Variation | | F-values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | caliper | | caliper RGR | | height | | volume | |
| | d.f. | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| Species (Spp) | 6 | 9.47*** | 3.21* | 1.55 | 5.50** | 1.36 | 9.22*** | 34.19*** | 7.04** |
| Fertilizer Rate (Fert) | 3 | 16.33*** | 3.54* | 13.69*** | 1.45 | 1.43 | 2.65 | 18.20*** | 6.78** |
| Spp × Fert | 18 | 1.57 | 1.12 | 1.50 | 0.58 | 1.12 | 0.64 | 1.15 | 1.44 |
| Block (Blk) | 4 | 4.33** | 0.50 | 4.18* | 0.50 | 4.60** | 1.45 | 6.11** | 0.53 |
| Blk × Spp | 24 | 1.21 | 1.44 | 1.27 | 1.41 | 0.74 | 1.05 | 0.70 | 1.58 |

*, $p \le 0.05$; **, $p \le 0.01$; ***, $p < 0.0001$

Table 2.2: Summary analysis of variance for specific leaf area (SLA), total dry weight, total leaf area (TLA), and canopy light interception for seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates.

| Source of Variation | d.f. | F-values SLA | dry wt | TLA | Interception† |
|---|---|---|---|---|---|
| Between subjects | | | | | |
| Species (Spp) | 6 | 108.38*** | 48.74*** | 32.02*** | 9.36*** |
| Fertilizer Rate (Fert) | 3 | 2.89* | 17.74*** | 14.64*** | 3.77* |
| Spp × Fert | 18 | 1.55 | 0.99 | 1.42 | 0.38 |
| Block (Blk) | 4 | 3.79* | 4.57** | 2.99* | 7.21*** |
| Blk × Spp | 24 | 2.08** | 1.08 | 1.27 | 1.51 |
| Within subjects | | | | | |
| Year (Yr) | 1 | 14.44** | 4.47* | 3.79 | |
| Spp × Yr | 5,6 | 9.10*** | 7.74*** | 5.63** | |
| Fert × Yr | 3 | 2.65 | 1.49 | 1.06 | |
| Spp × Fert × Yr | 15,18 | 1.04 | 1.30 | 1.06 | |
| Blk × Spp × Fert | 82 | 1.02 | 0.63 | 0.50 | |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

† canopy light interception measured on 23 July 2007

Table 2.3: Summary analysis of variance for photosynthesis ($A_{max}$), conductance ($g_{wv}$), and transpiration (E) for seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates.

| Source of Variation | 2006 F-values | | | | 2007 F-values | | | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | $A_{max}$ | $g_{wv}$ | WUE | d.f. | $A_{max}$ | $g_{wv}$ | WUE |
| Between subjects | | | | | | | | |
| Species (Spp) | 6 | 15.13*** | 13.27*** | 6.56** | 6 | 14.71*** | 8.74*** | 0.88 |
| Fertilizer Rate (Fert) | 3 | 0.57 | 3.17* | 2.06 | 3 | 4.79** | 26.15*** | 15.48*** |
| Spp × Fert | 18 | 1.07 | 1.42 | 1.22 | 18 | 1.63 | 1.15 | 1.00 |
| Block (Blk) | 4 | 1.91 | 2.02 | 4.76** | 4 | 6.96** | 5.40** | 4.59** |
| Blk × Spp | 24 | 1.65* | 2.40** | 2.26** | 24 | 0.98 | 1.10 | 1.14 |
| Within subjects | | | | | | | | |
| Date (D) | 3 | 73.54*** | 185.54*** | 82.06*** | 4 | 19.86*** | 197.17*** | 164.62*** |
| Spp × D | 18 | 10.48*** | 12.79*** | 9.36*** | 24 | 10.31*** | 14.42*** | 5.62*** |
| Fert × D | 9 | 1.33 | 0.78 | 0.61 | 12 | 2.62** | 2.86** | 1.23 |
| Spp × Fert × D | 54 | 0.80 | 0.47 | 0.61 | 72 | 0.81 | 0.97 | 0.59 |
| Blk × Spp × Fert | 83 | 0.70 | 0.56 | 0.49 | 82 | 1.00 | 1.17 | 0.80 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.4. Pearson's correlation values for total height growth, total caliper growth, total volume growth, specific leaf area (SLA), total leaf area (TLA), canopy light interception (intercept), photosynthetic rate ($A_{max}$), conductance ($g_{wv}$), relative chlorophyll content (SPAD), leaf nitrogen concentration (N) and pH for seven taxa of deciduous shade tree; and nitrate-N ($NO_3$) in pour-thru leachate of *Ulmus* Triumph™, *Acer rubrum*, and *Platanus* x *acerifolia* grown in a PIP production system under four fertilizer levels.

| | caliper | volume | SLA | TLA | Intercept | $A_{max}$ | $g_{wv}$ | SPAD | N | pH | $NO_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| height | 0.04 | 0.15** | -0.29*** | -0.01 | 0.16** | 0.03 | -0.05 | 0.21*** | 0.32*** | -0.22** | 0.17* |
| caliper | | 0.89*** | 0.25*** | 0.26*** | -0.10* | -0.24*** | -0.15** | -0.02 | 0.21*** | -0.12 | 0.11 |
| volume | | | 0.30*** | 0.33*** | -0.14** | -0.21*** | -0.15** | -0.03 | 0.15** | -0.14 | 0.14* |
| SLA | | | | 0.40*** | -0.15*** | -0.20*** | -0.11** | -0.12*** | 0.07* | 0.05 | -0.04 |
| TLA | | | | | 0.14** | -0.21*** | -0.16*** | 0.03 | 0.13*** | -0.02 | 0.20*** |
| Intercept | | | | | | 0.02 | 0.17*** | 0.47*** | 0.04 | 0.43*** | 0.04 |
| $A_{max}$ | | | | | | | 0.75*** | 0.13*** | -0.28*** | 0.16** | -0.25*** |
| $g_{wv}$ | | | | | | | | 0.17*** | -0.26*** | 0.26*** | -0.27*** |
| SPAD | | | | | | | | | -0.04 | 0.10* | 0.17*** |
| N | | | | | | | | | | -0.19*** | 0.39*** |
| pH | | | | | | | | | | | -0.32*** |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.5. Summary analysis of variance for effects of predawn and midday stem water potential ($\Psi_W$) for seven taxa of landscape shade tree grown in a PIP production system, fertilized with either 1.32 g fertilizer $L^{-1}$ or 5.26 g fertilizer $L^{-1}$ in 2007.

| Source of variation | d.f. | F-values predawn | F-values midday |
|---|---|---|---|
| Between subjects | | | |
| Species (Spp) | 6 | 16.85*** | 14.96*** |
| Fertilizer Rate (Fert) | 1 | 0.29 | 6.37* |
| Spp × Fert | 6 | 0.19 | 1.13 |
| Block (Blk) | 4 | 4.93** | 5.79** |
| Blk × Spp | 24 | 1.40 | 1.50 |
| Within subjects | | | |
| Date (D) | 4 | 71.89*** | 53.44*** |
| Spp × D | 24 | 3.28*** | 2.50** |
| Fert × D | 4 | 0.62 | 1.75 |
| Spp × Fert × D | 24 | 1.31 | 1.95** |
| Blk × Spp × Fert | 28 | 1.84** | 0.95 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.6: Summary analysis of variance for foliar nutrient concentrations in mid-season leaf samples (collected 3 September 2006 and 31 July 2007) from seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates.

| Source of variation | | F-values | | |
|---|---|---|---|---|
| | d.f. | N (mg/L) | P (mg/L) | K (mg/L) |
| Between subjects | | | | |
| Species (Spp) | 6 | 40.33*** | 16.52*** | 13.44*** |
| Fertilizer Rate (Fert) | 3 | 219.13*** | 25.67*** | 21.13*** |
| Spp × Fert | 18 | 2.05* | 1.95* | 0.87 |
| Block (Blk) | 4 | 5.42** | 2.01 | 1.34 |
| Blk × Spp | 24 | 0.94 | 1.20 | 0.79 |
| Within subjects | | | | |
| Date (D) | 1 | 378.77*** | | |
| Spp × D | 6 | 11.04*** | | |
| Fert × D | 3 | 14.32*** | | |
| Spp × Fert × D | 18 | 2.35** | | |
| Blk × Spp × Fert | 83 | 1.42* | | |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.7. Percent nitrogen retranslocation (%N), comparing N concentration in mid-season leaf samples ($N_{mid}$; collected 3 September) to N concentration in litterfall leaf samples ($N_{litt}$) for seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates in 2006. Percent retranslocation calculated as $[((N_{mid} - N_{litt})/N_{mid})*100]$.

| Source of variation | | F-values |
|---|---|---|
| | d.f. | %N |
| Species (Spp) | 6 | 22.43*** |
| Fertilizer Rate (Fert) | 3 | 3.98* |
| Spp × Fert | 18 | 2.32** |
| Block (Blk) | 4 | 0.76 |
| Blk × Spp | 23 | 4.39*** |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.8. Summary analysis of variance for effects of relative chlorophyll content (SPAD) and chlorophyll fluorescence ($F_v/F_m$) measurements for seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates.

| Source of variation | | 2006 F-values | | 2006 F-values | | 2007 F-values | | 2007 F-values |
|---|---|---|---|---|---|---|---|---|
| | d.f. | SPAD | d.f. | $F_v/F_m$ | d.f. | SPAD | d.f. | $F_v/F_m$ |
| Between subjects | | | | | | | | |
| Species (Spp) | 6 | 24.80*** | 6 | 16.93*** | 6 | 53.24*** | 6 | 14.05*** |
| Fertilizer Rate (Fert) | 3 | 0.74 | 3 | 0.98 | 3 | 95.56*** | 3 | 0.62 |
| Spp × Fert | 18 | 1.02 | 18 | 1.40 | 18 | 2.04* | 18 | 1.81** |
| Block (Blk) | 4 | 0.53 | 4 | 0.71 | 4 | 0.57 | 4 | 0.72 |
| Blk × Spp | 24 | 1.47 | 24 | 1.08 | 24 | 1.87* | 24 | 1.26 |
| Within subjects | | | | | | | | |
| Date (D) | 3 | 71.60*** | 2 | 60.77*** | 4 | 412.79 | 2 | 70.83*** |
| Spp × D | 18 | 5.74*** | 12 | 2.00* | 24 | 15.47 | 12 | 7.38*** |
| Fert × D | 9 | 0.83 | 6 | 1.49 | 12 | 2.98 | 6 | 1.03 |
| Spp × Fert × D | 54 | 0.85 | 36 | 0.68 | 72 | 1.53 | 36 | 0.58 |
| Blk × Spp × Fert | 83 | 1.28 | 83 | 0.84 | 82 | 2.33 | 82 | 0.790 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table 2.9: Summary analysis of variance for $NO_3$, pH, and EC concentrations in PourThru leachate samples collected in 2006 and 2007 for *Platanus* x *acerifolia*, *Acer rubrum* 'Franksred', and *Ulmus* Triumph™ grown in a PIP system under four fertilizer rates.

| Source of variation | | F-values | | |
|---|---|---|---|---|
| | d.f. | $NO_3$ | pH | EC |
| Between subjects | | | | |
| Species (Spp) | 2 | 0.23 | 3.98 | 0.96 |
| Fertilizer Rate (Fert) | 3 | 80.52*** | 26.20*** | 98.70*** |
| Spp × Fert | 6 | 1.14 | 1.33 | 1.19 |
| Block (Blk) | 4 | 0.46 | 6.37* | 3.57 |
| Blk × Spp | 8 | 1.31 | 2.81* | 1.19 |
| Within subjects | | | | |
| Date (D) | 9 | 35.51*** | 73.20*** | 69.19*** |
| Spp × D | 18 | 0.66 | 3.73*** | 1.24 |
| Fert × D | 27 | 8.24*** | 1.67* | 3.48*** |
| Spp × Fert × D | 54 | 0.99 | 0.78 | 1.08 |
| Blk × Spp × Fert | 36 | 1.48 | 1.34 | 1.57* |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

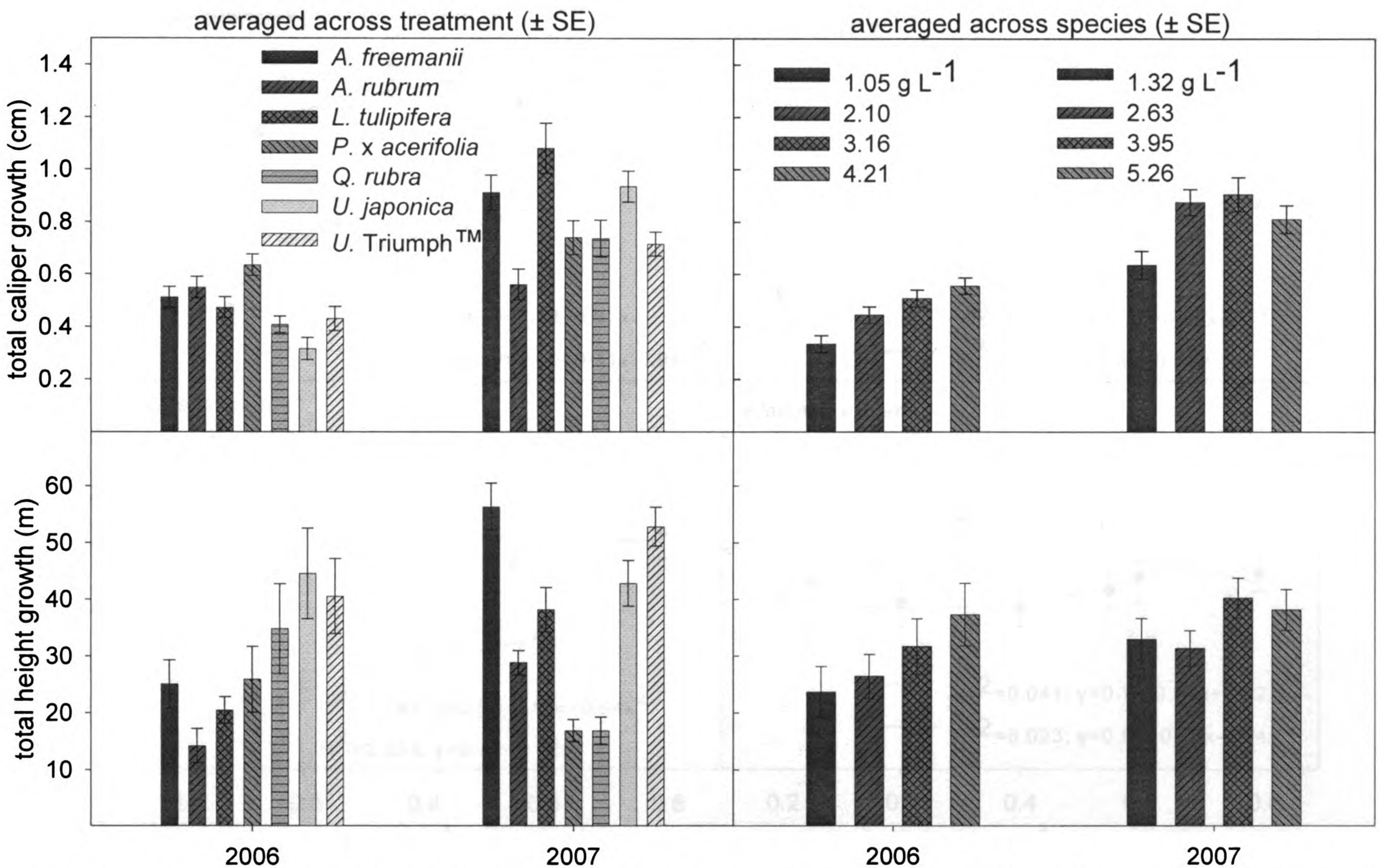



Figure 2.1. Species and fertilizer effects on total seasonal caliper (cm) growth and total seasonal height (cm) growth (± SE) in 2006 and 2007 for seven taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer levels.

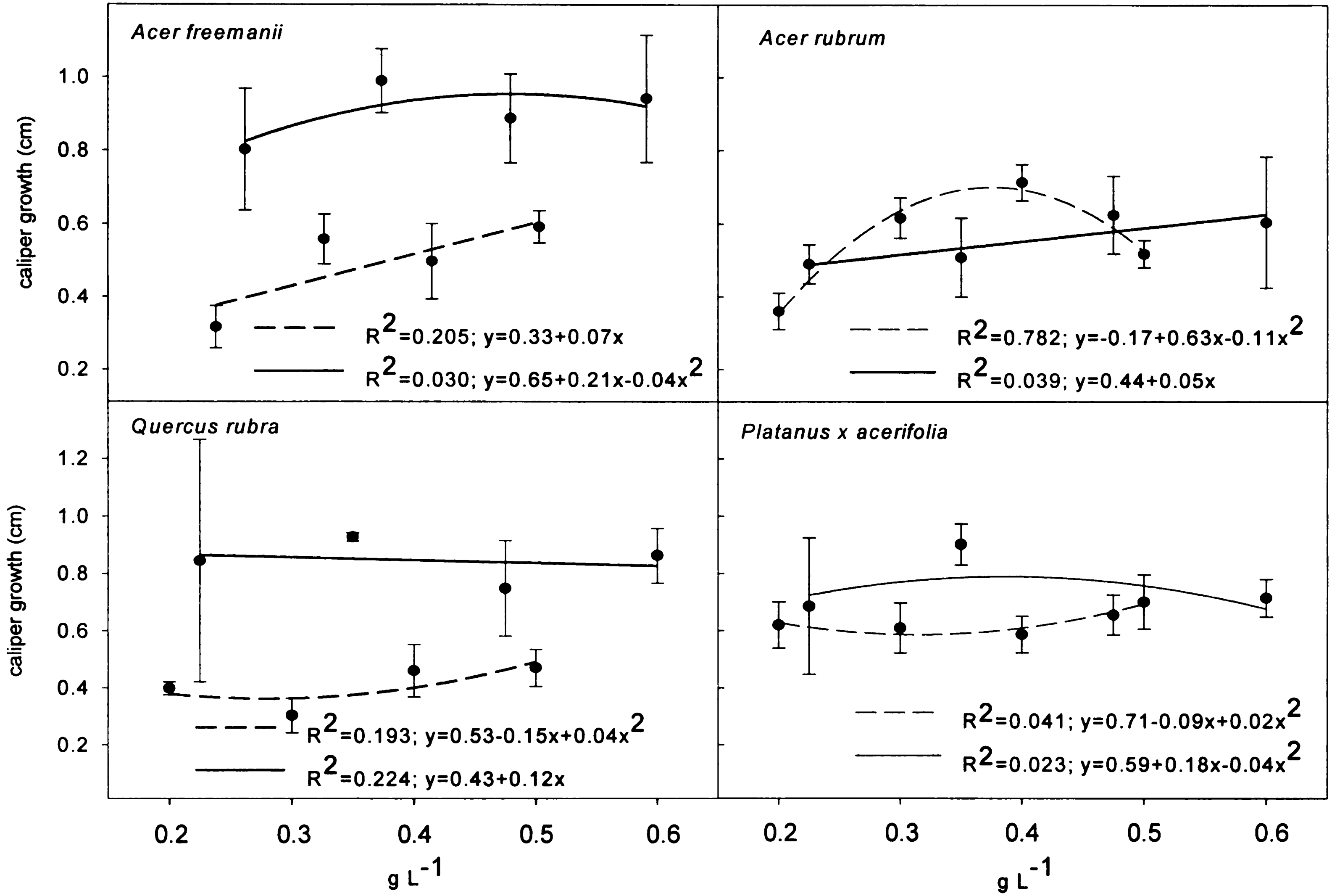



Figure 2.2a. Caliper growth response (± SE) to fertilizer treatment ($g \cdot L^{-1}$ container) for four taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer levels. Dashed lines represent 2006 data and solid lines represent 2007.

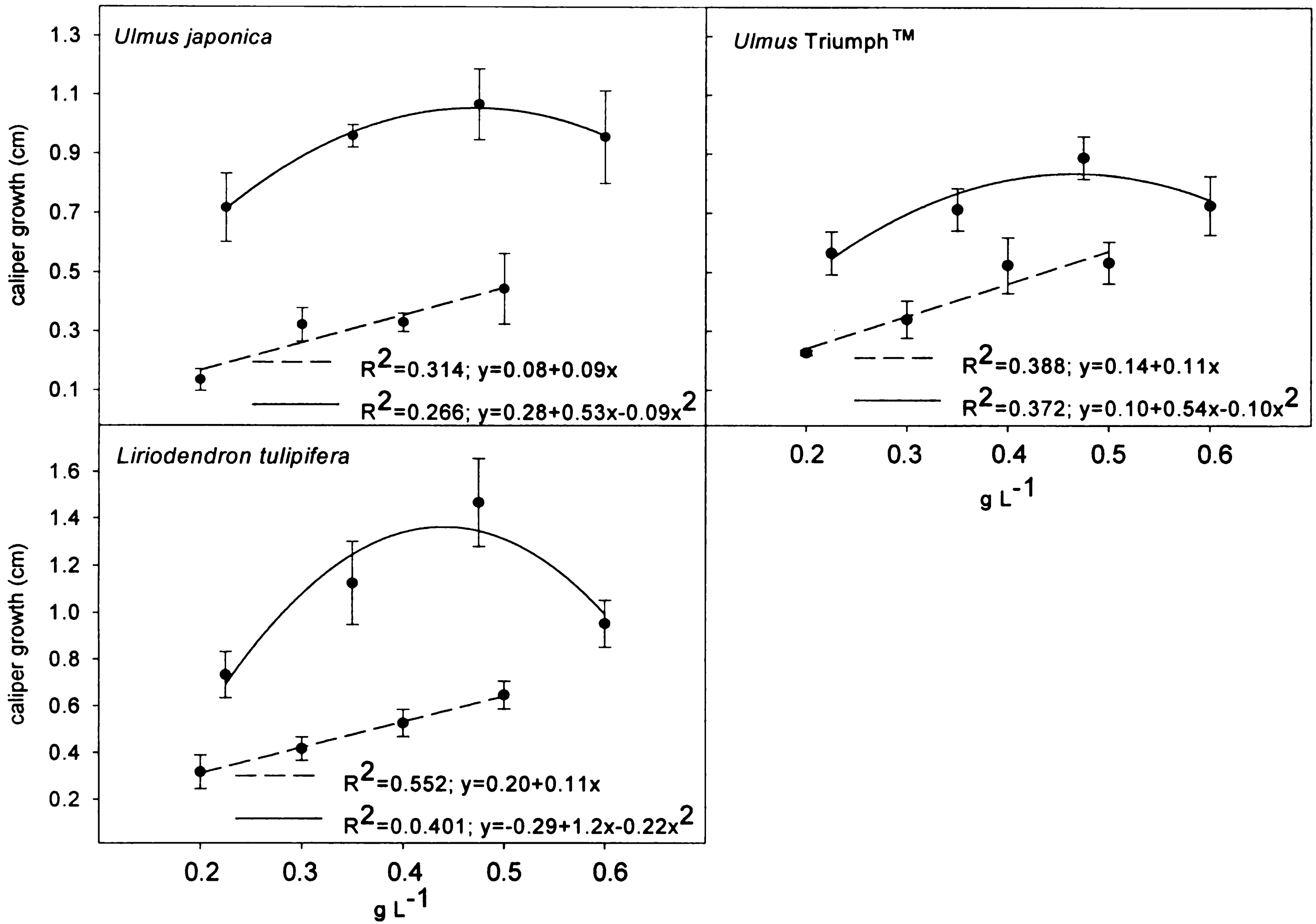



Figure 2.2b. Caliper growth response (± SE) to fertilizer treatment ($g \cdot L^{-1}$ container) for three taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer levels. Dashed lines represent 2006 data and solid lines represent 2007.

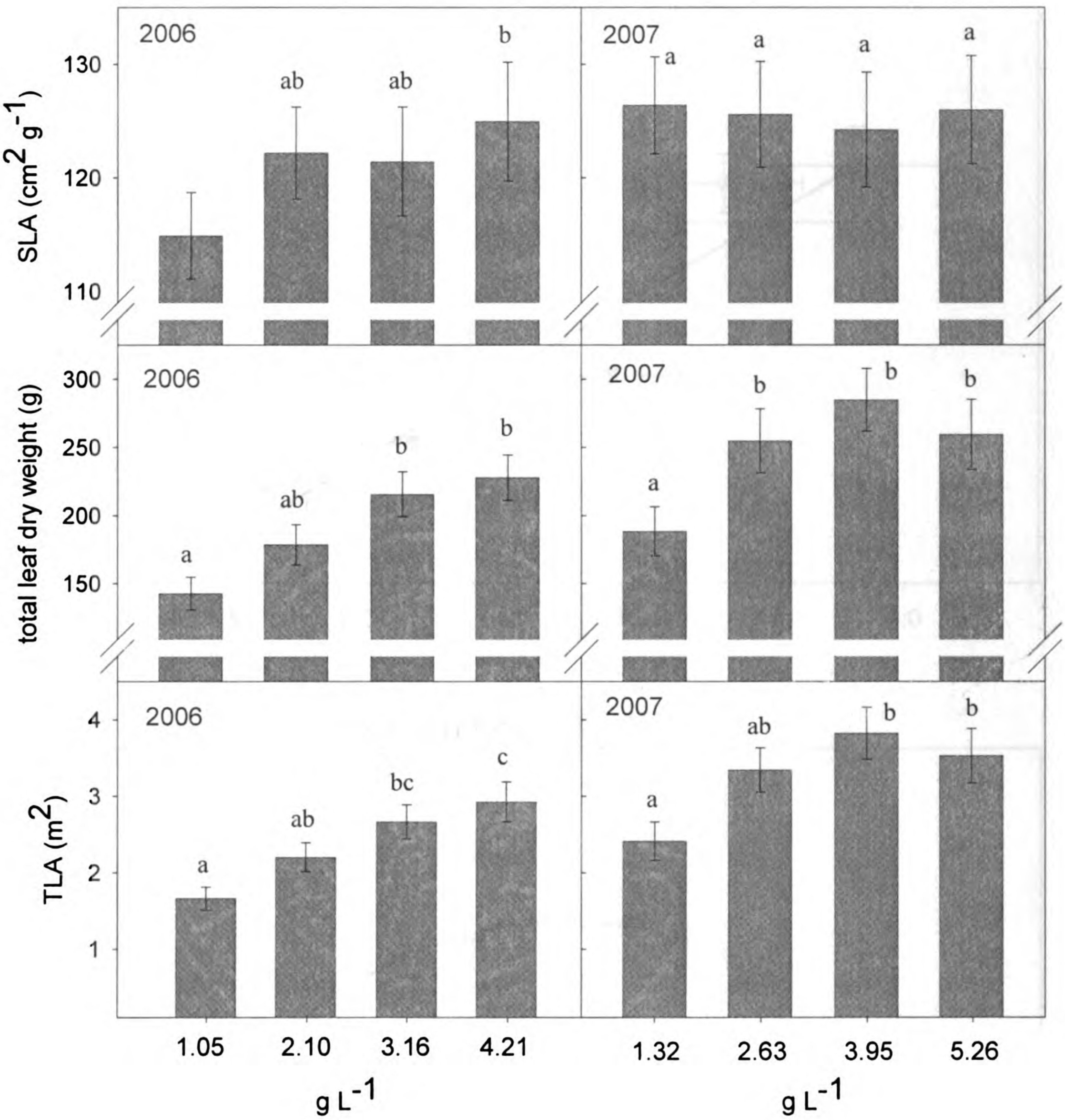



Figure 2.3. Specific leaf area (SLA), total leaf dry weight, and total leaf area (TLA) (± SE) for seven taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer levels in 2006 and 2007. Different letters indicate means are significantly different using PROC lsmeans with Tukey's adjustment, $p \leq 0.05$.

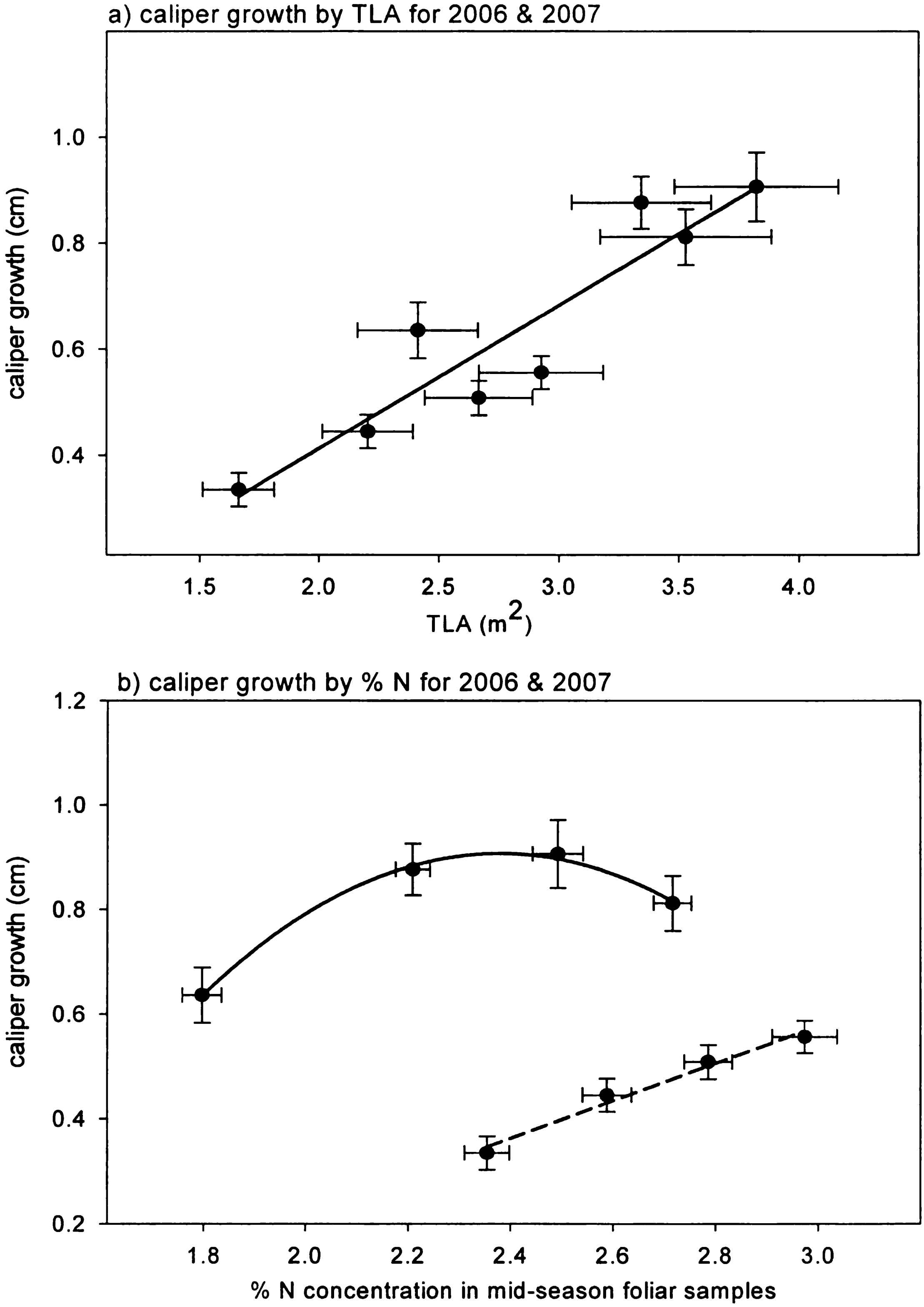



Figure 2.4. Caliper growth (cm) (± SE) in relation to a) total leaf area (TLA; $m^2$) (± SE); and b) percent foliar N concentration (± SE) for seven taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer levels.

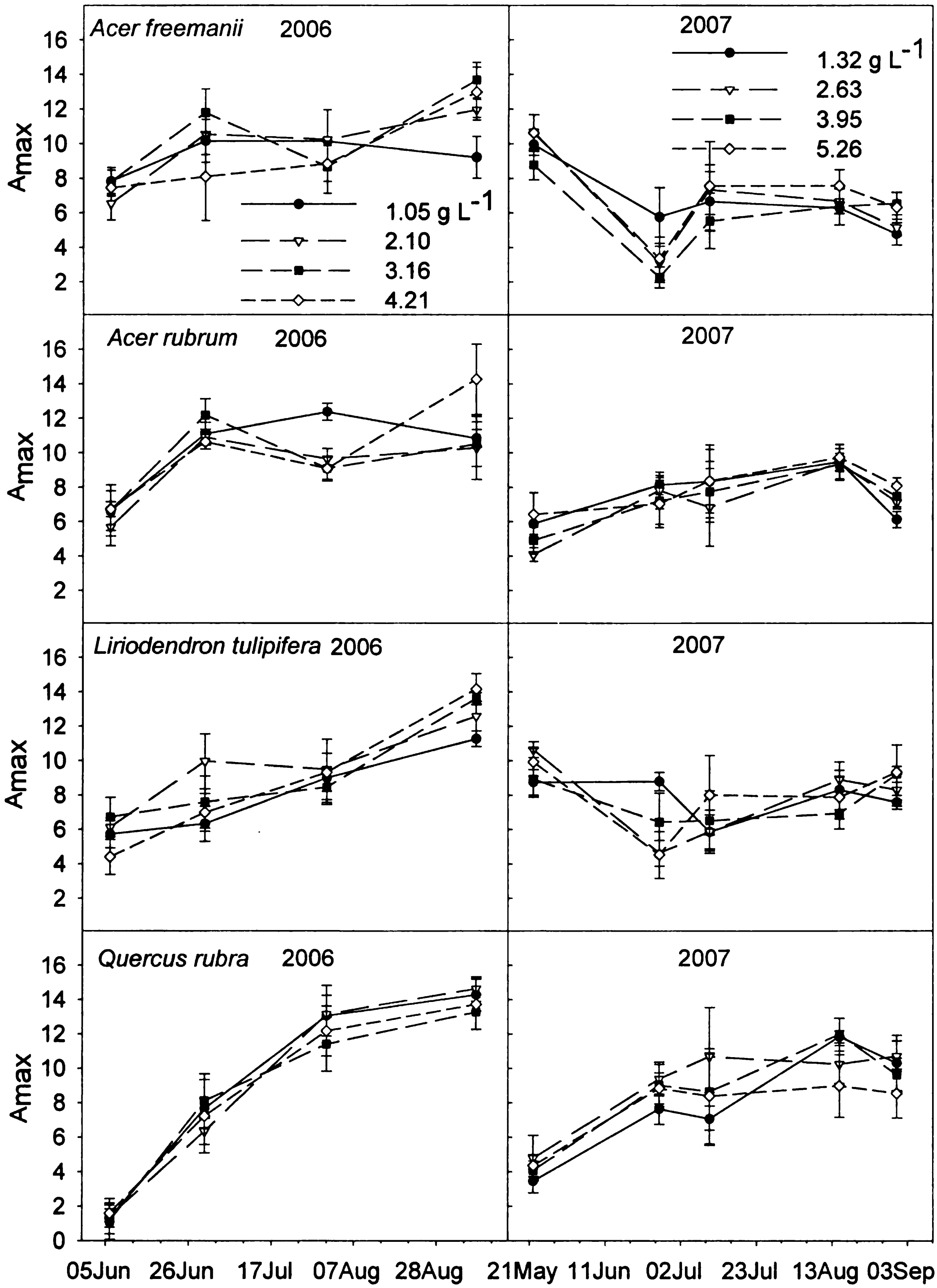



Figure 2.5a. Net photosynthesis rates ($A_{max}$; µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) (± SE) for four taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization.

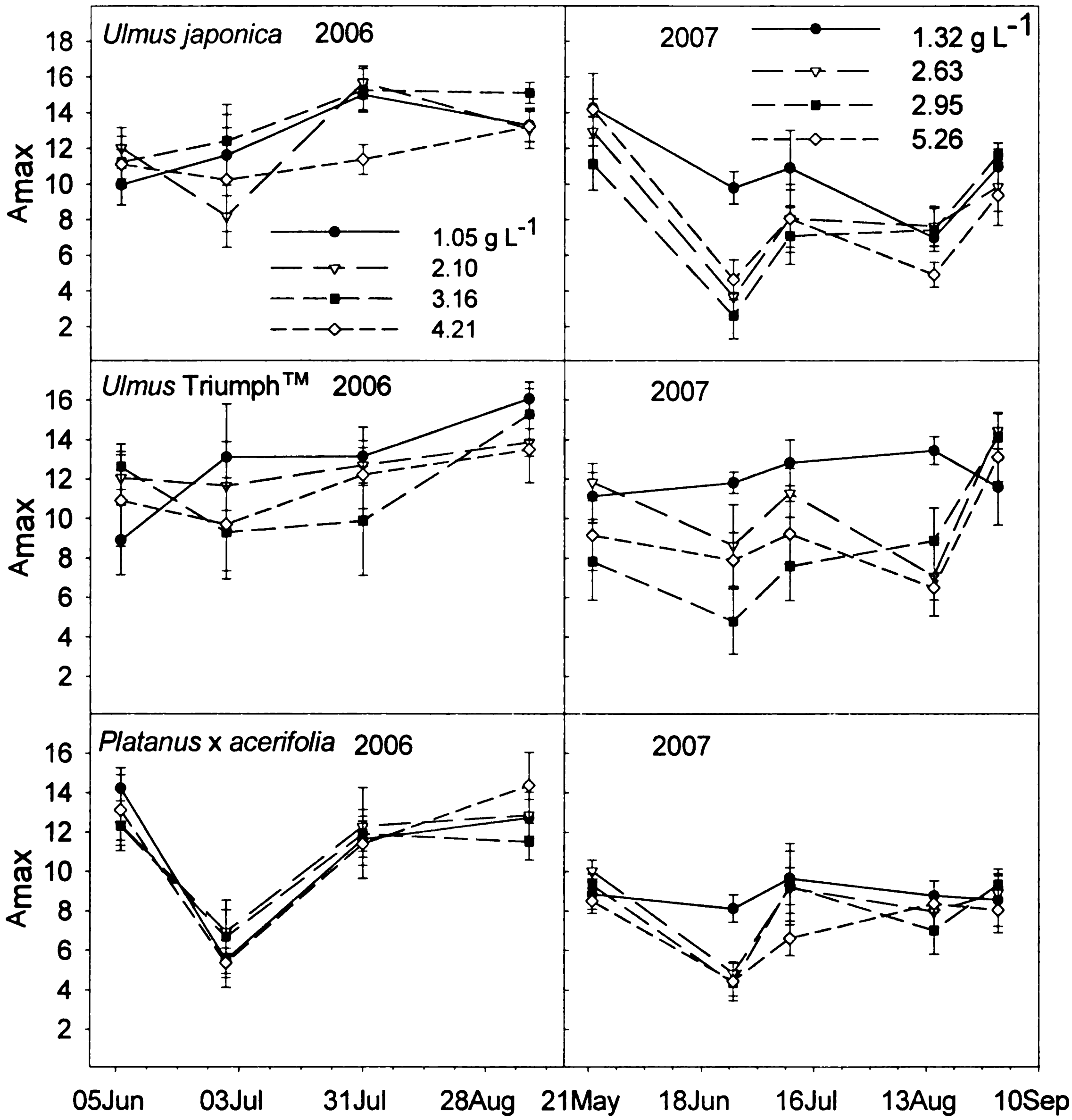



Figure 2.5b. Net photosynthesis rates ($A_{max}$; μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) (± SE) for three taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization.

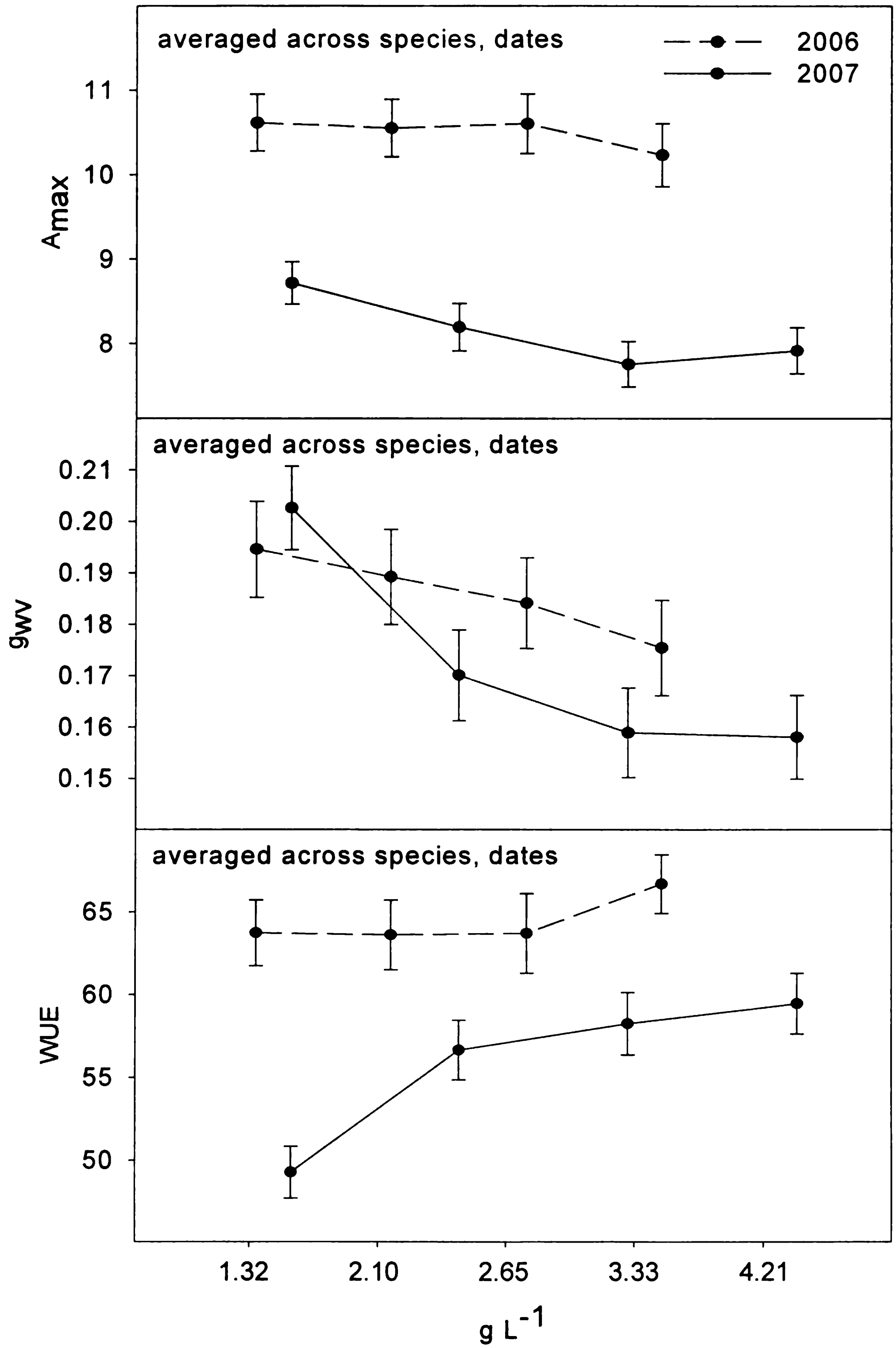



Figure 2.6. Net photosynthesis ($A_{max}$; µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), leaf conductance ($g_{wv}$; mol $H_2O \cdot m^{-2} \cdot s^{-1}$), and intrinsic water use efficiency (WUE; $A_{max}/g_{wv}$) (± SE) for seven taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization.

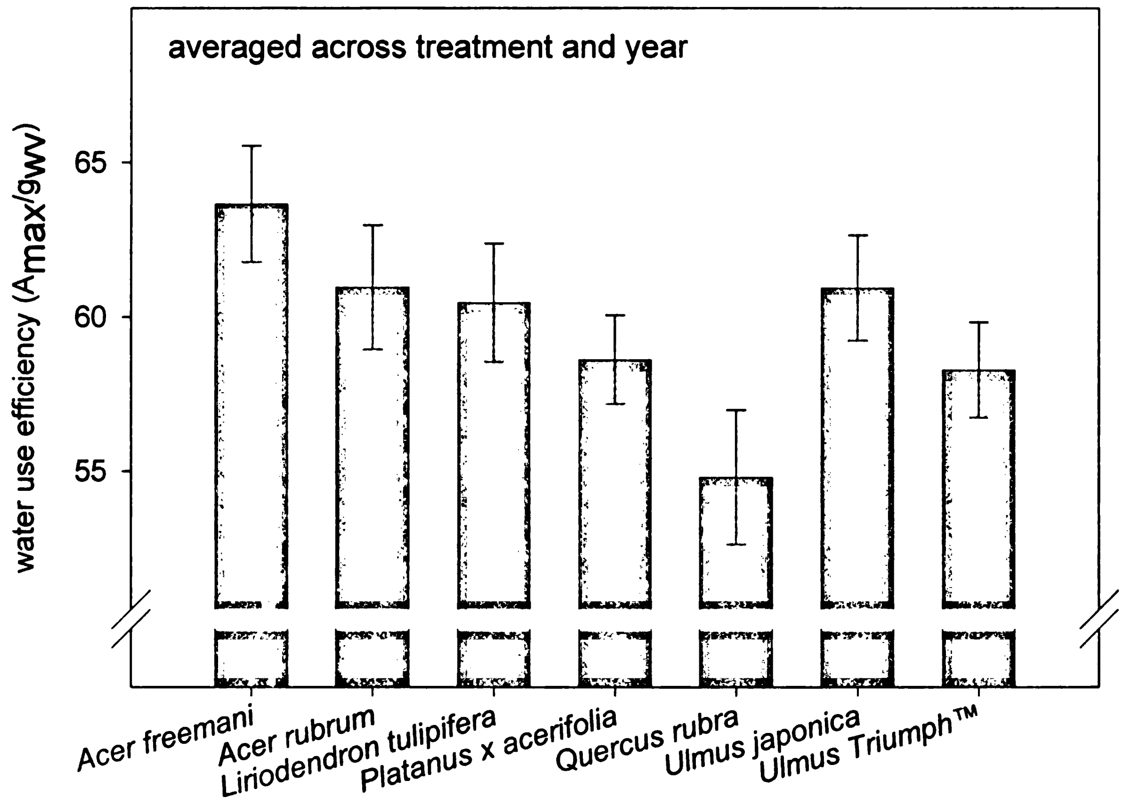



Figure 2.7. Intrinsic water use efficiency (WUE; $A_{max}/g_{wv}$) (± SE) for seven taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization.

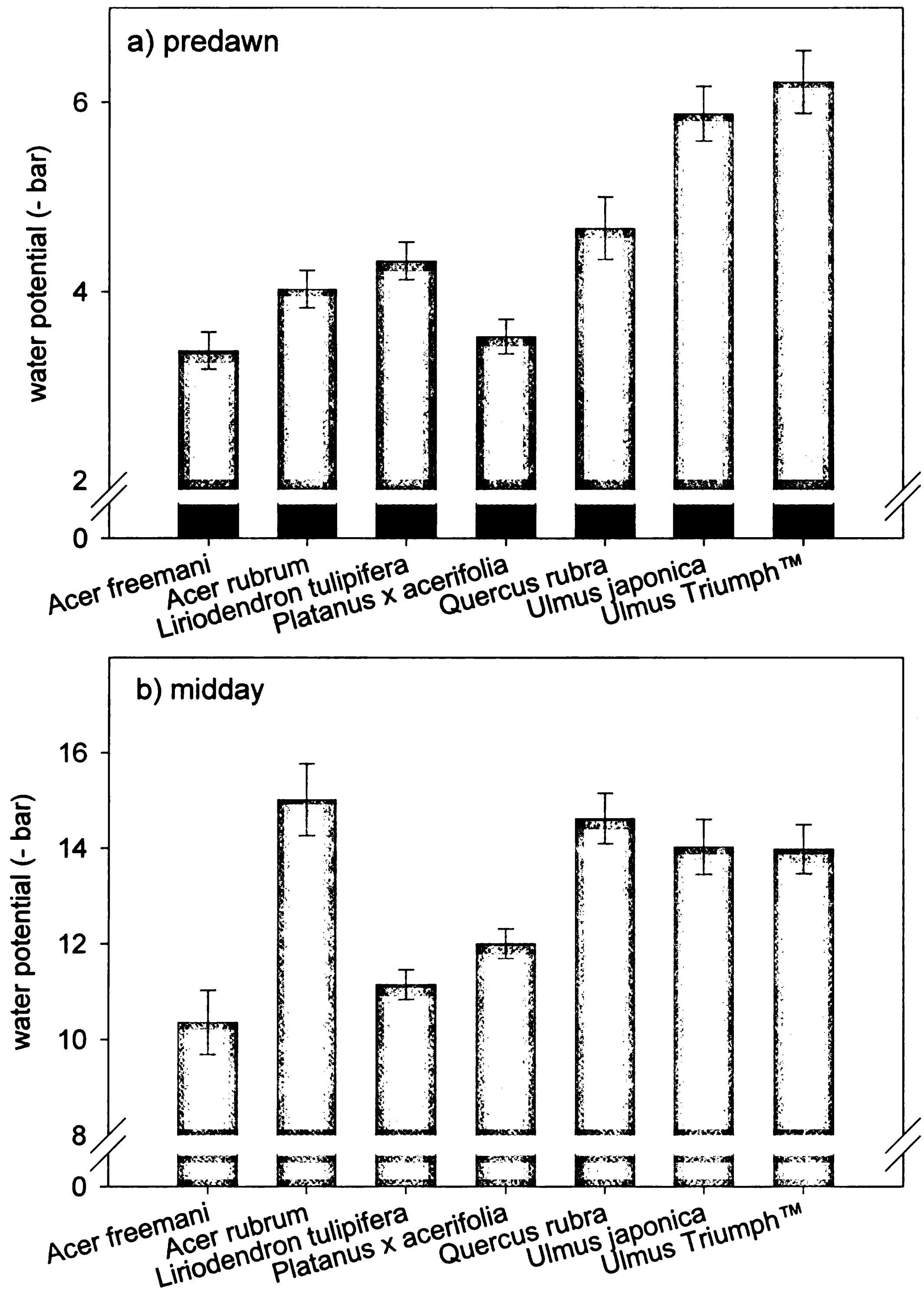



Figure 2.8. a) Predawn and b) midday stem water potential ($\Psi_w$; - bar) (± SE) for seven taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization (data averaged across treatment level).

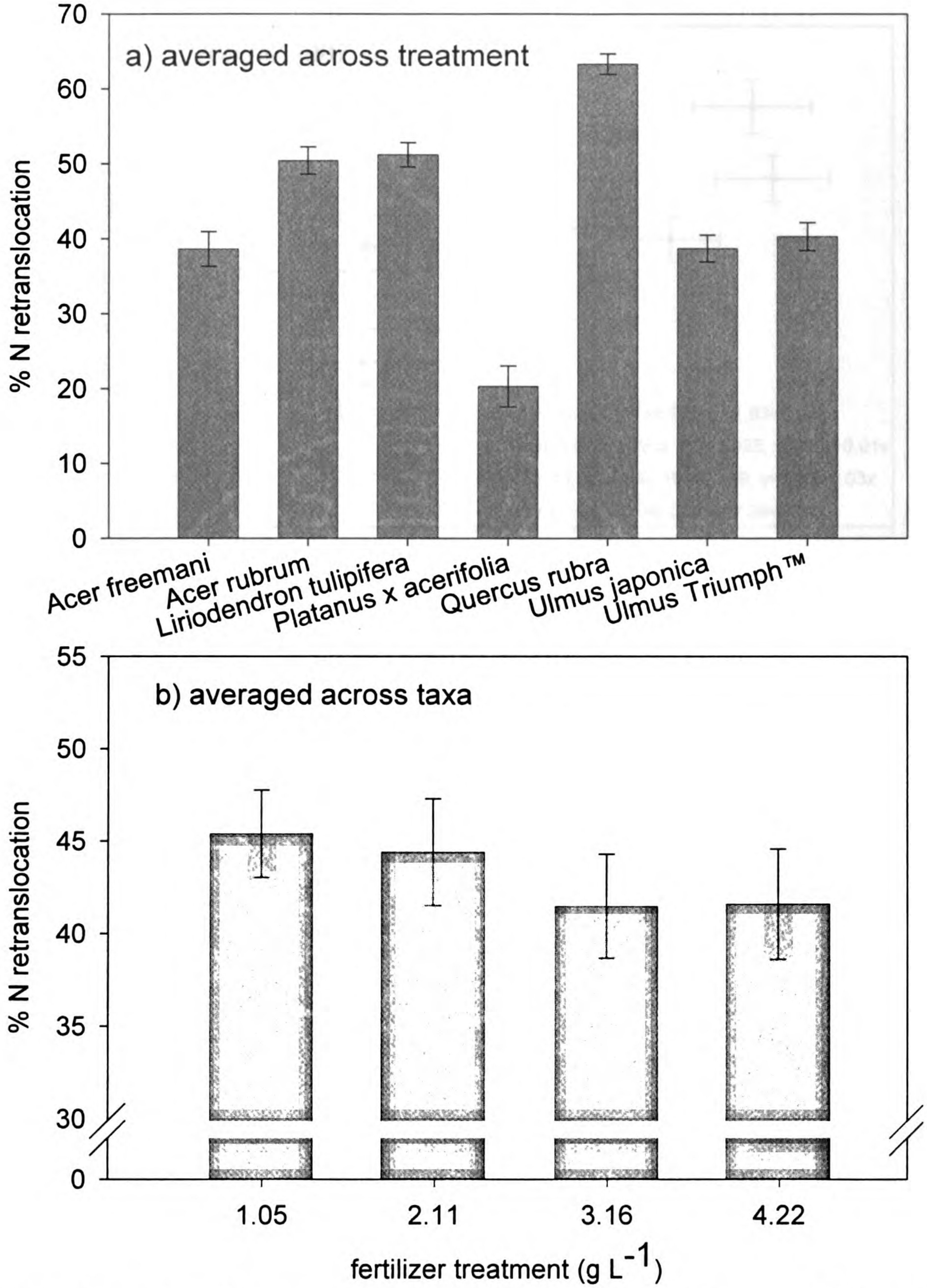
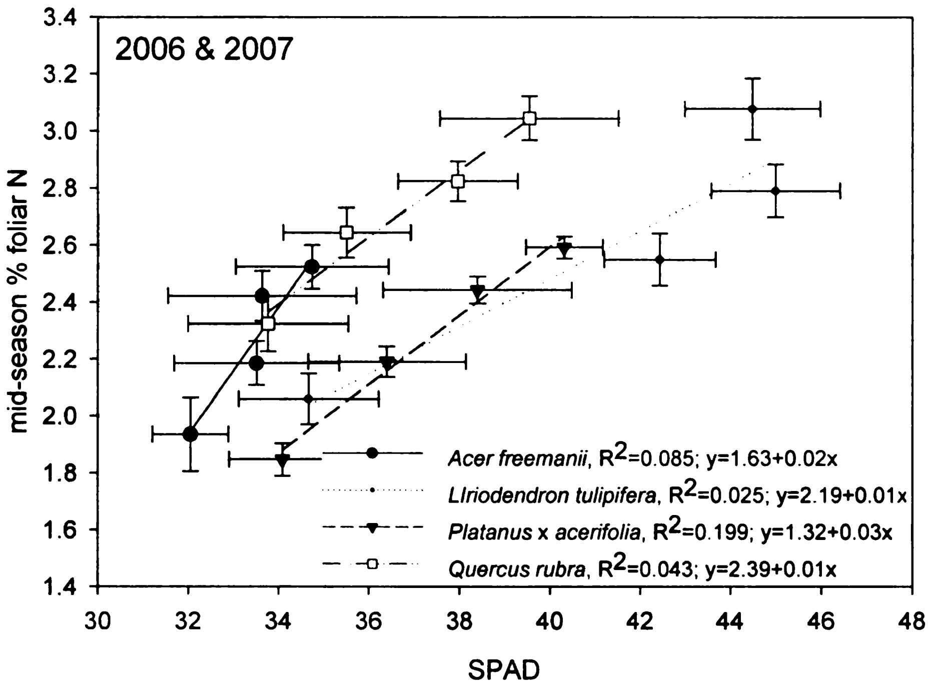



Figure 2.9. Percent N retranslocation [(($N_{midseason} - N_{litterfall}$)/$N_{midseason}$)*100] (± SE) for 2006, estimating differences between N concentration in litterfall leaves and N concentration in mid-season foliar samples for seven taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization. Comparisons between a) taxa, when averaged across treatment level, and b) treatment, when averaged across all seven taxa.



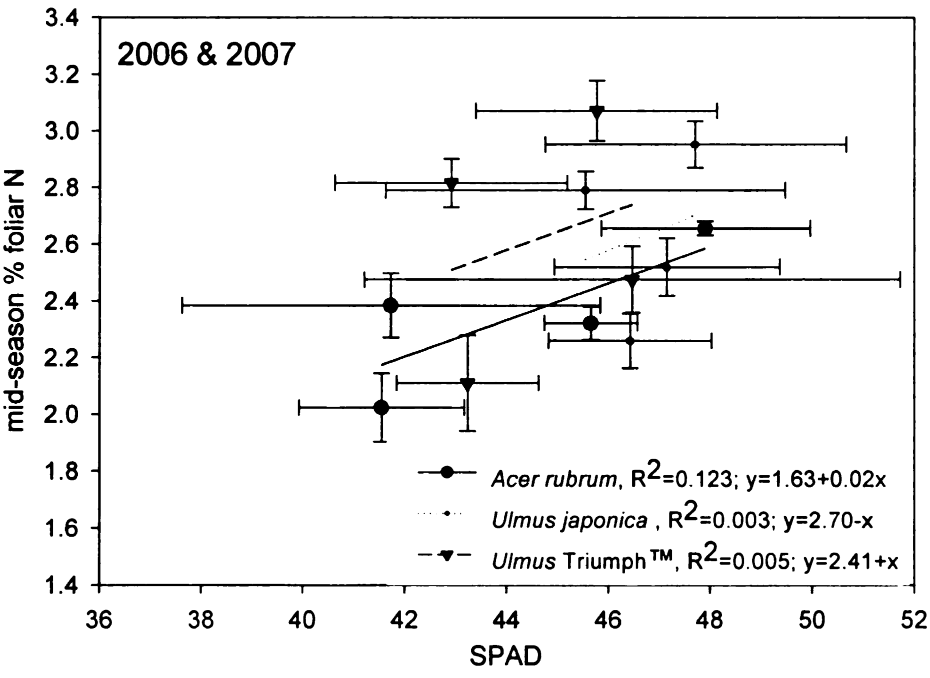




Figure 2.10. Relationships between foliar N concentrations and SPAD values for seven taxa of landscape shade tree grown in a pot-in-pot production system under four levels of fertilization.

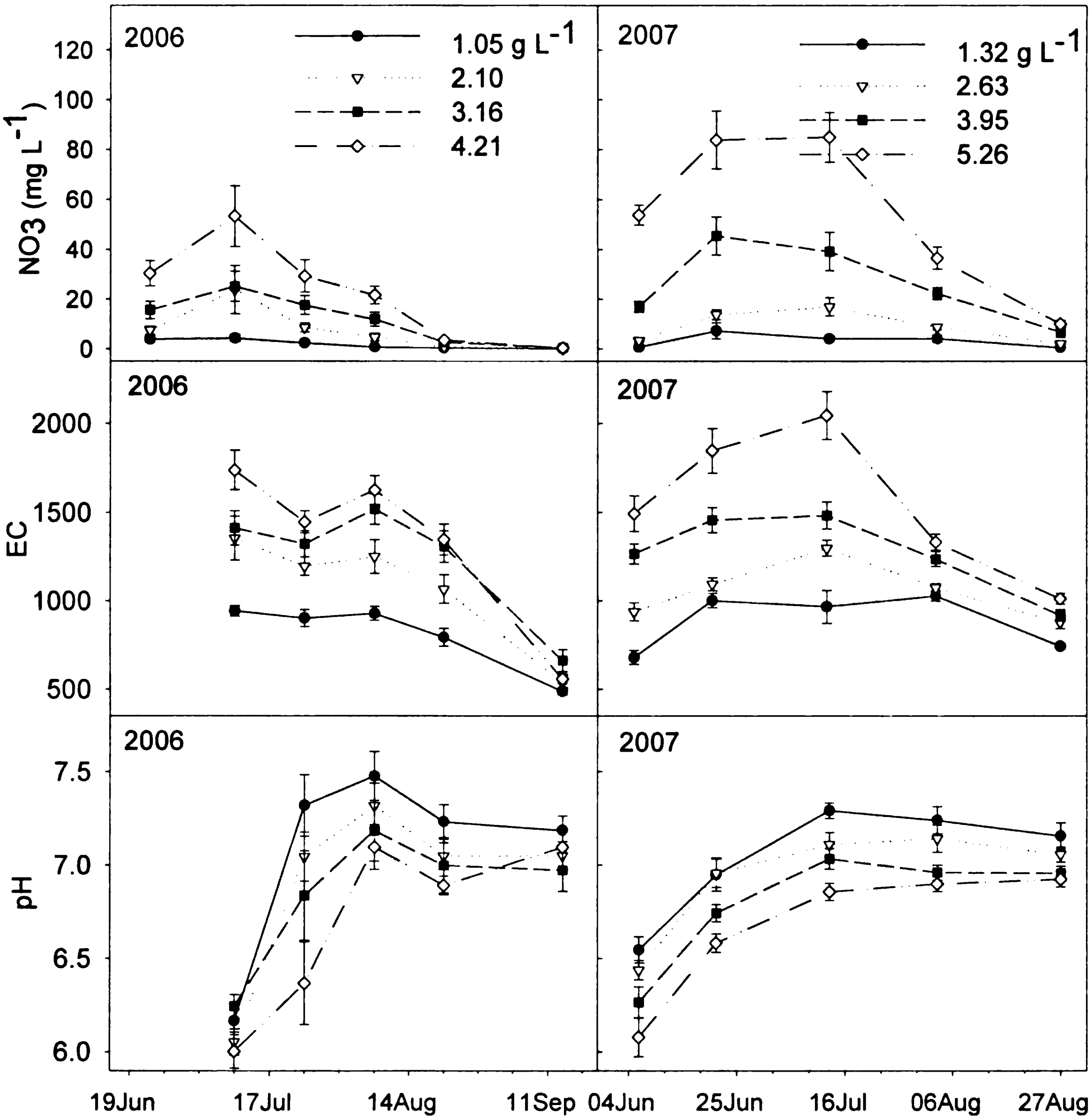



Figure 2.11. Nitrate-N concentration, EC, and pH (± SE) of PourThru leachate samples collected in 2006 and 2007 for *Ulmus* Triumph™, *Acer rubrum* 'Franksred', *Platanus* × *acerifolia* 'Bloodgood' grown under four levels of fertilization. Data averaged across taxa.

## Literature Cited

Adrian, J.L., C.C. Montgomery, B.K. Behe, P.A. Duffy, and K.M. Tilt. 1998. Cost comparisons for infield, above ground container and pot-in-pot production systems. J. Environ. Hort. 16(2):65-68.

Allen, H.L., T.R. Fox, and R.G. Campbell. 2005. What is ahead for intensive pine plantation silviculture in the South? South. J. Appl. For. 29(2):62-69.

Altland, J. 2006. Foliar chlorosis in field-grown red maples. HortScience. 41(5):1347-1350.

Bilderback, T.E. 2001. Using the pourthru procedure for checking EC and pH for nursery crops. NC State University Horticulture Information Leaflet 450. 5 pp.

Bogart, K. 2006. Three most common methods – measuring vine water status. Viticulture and Enology Trellis Alliance, Univ. Calif. Davis. 21 May 2007. http://wineserver.ucdavis.edu/pdf/attachment/vine%20water%20status.pdf

Cregg, B.M., M.W. Duck, C.M. Rios, D.B. Rowe, and M.R. Koelling. 2004. Chlorophyll fluorescence and needle chlorophyll concentration of fir (*Abies* sp.) seedlings in response to pH. HortScience. 39(5):1121-1125.

Daughtry, C.S.T., C.L. Walthall, M.S. Kim, E.B. de Colstoun, and J.E. McMurtre. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sensing Environ. 74:229-239.

Davenport, J.R., E.M. Perry, N.S. Lang, and R.G. Stevens. 2005. Leaf spectral reflectance for nondestructive measurement of plant nutrient status. HortTech. 15(1):31-35.

Fain, G.B., K.M. Tilt, C.H. Gilliam, H.G. Ponder and J.L. Sibley. 1998. Effects of cyclic micro-irrigation and substrate in pot-in-pot production. J.Environ.l Hort. 16:215-218.

Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2007. Tree nutrition and forest fertilization of pine plantations in the southern United States. South. J. Appl. For. 31(1):5-11.

Fritschi, F.B. and J.D. Ray. 2007. Soybean leaf nitrogen, chlorophyll content, and chlorophyll *a/b* ratio. Photosynthetica. 45(1):92-98.

Fulcher, A., W. Dumwell, R. McNiel, D. Wolfe, and L. Murdock. 2004. Effect of fertilizer rate on growth of seven tree species in pot-in-pot production. Univ. of Kentucky Agricultural Experiment Station. Nursery and landscape program research report. pr502. pp. 7-8.

Haase, D.L. and R. Rose. 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. Forest Science. 41(1):54-66.

Halcomb, M.A. and D.C. Fare. 1995. A survey of the pot-in-pot growers in middle Tennessee. Proc. Southern Nursery Assoc. Res. Conf. 40:147-148.

Huang, J., X. Wang, and E. Yan. 2006. Leaf nutrient concentration, nutrient resorption and litter decomposition in an evergreen broad-leaved forest in eastern China. For. Ecol. and Management. 239:150-158.

Huffman, S.A. and K.A. Barbarick, 1981. Soil nitrate analysis by cadmium reduction. Communications in Soil Science and Plant Analysis. 12(1):79-89.

Landis, T.D. and E. van Steenis. 2003. Macronutrients – nitrogen: part 1. For. Nurs. Notes. Summer. 5 pp.

Loh, F.C.W., J.C. Grabosky, and N.L. Bassuk. 2002. Using the SPAD 502 meter to assess chlorophyll and nitrogen content of Benjamin fig and cottonwood leaves. HortTech. 12:682-686.

Luyssaert, S, J. Staelens, A.D. Schrijver. 2005. Does the commonly used estimator of nutrient resorption in tree foliage actually measure what it claims to? Oecologia. 144: 177-186.

Mathers, H. 2000. Pot-in-pot container culture. The Nursery Papers. 2:1-6.

MAWN. 2007. Michigan Automated Weather Network. 21 May 2008. http://www.agweather.geo.msu.edu/mawn/.

NASS. 2007a. Nursery crops summary 2006. National Agricultural Statistics Service and United States Department of Agriculture. 18 January 2008. http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plants

NASS. 2007b. Oregon Nursery and Greenhouse Survey 2006. National Agricultural Statistics Service and United States Department of Agriculture. 18 January 2008. http://www.nass.usda.gov/Statistics_by_State/Oregon/Publications/Horticulture/Nursery2007final.pdf

Neal, C. 2004. Production Systems for Small Trees and Shrubs in New Hampshire. University of New Hampshire Cooperative Extension. 17 December 2007. http://extension .unh.edu/agric/AGNLT/PSSTSNH.htm

Nzokou, P., N.J. Gooch, and B.M. Cregg. 2007. Survivability of containerized live Christmas trees after indoor display. Great Lakes Christmas Tree J. 2(4):4-9.

Parent, L.E., L. Khiari, and A. Pettigrew. 2005. Nitrogen diagnosis of Christmas tree needle greenness. Can. J. Plant Sci. 85:939-947.

Ritchie, G.A. 2006. Chlorophyll Fluorescence: What Is It and What Do the Numbers Mean? USDA Forest Service Proceedings RMRS-P-43. 34-43.

Roberts, D.R. 1993. How Pot-in-Pot systems save time, money. Nursery Manager 9(6):46-50.

Ruter, J.M. 1998a. Fertilizer rate and pot-in-pot production increase growth of Heritage river birch. J. Environ. Hort. 16(3):135-138.

Ruter, J. M. 1998b. Pot-in-pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. J. Environ. Hort. 16:159-162.

Ruter, J. M., 1997. The practicality of pot-in-pot. American Nurseryman. 185(1):32-37.

Ruter, J.M. 1993. Field performance of two species as influenced by container production system. Proc. Southern Nursery Assoc. Res. Conf. 38:150-152.

Shackel, K.A., H. Ahmadi, W. Biasi, R. Buchner, D. Goldhamer, S. Gurusinghe, J. Hasey, D. Kester, B. Krueger, B. Lampinen, G. McGourty, W. Micke, E. Mitcham, B. Olson, K. Pelletrau, H. Philips, D. Ramos, L. Schwankl, S. Sibbett, R. Snyder, S. Southwick, M. Stevenson, M. Thorpe, S. Wienbaum, and J. Yeager. 1997. Plant water status as an index of irrigation need in deciduous fruit trees. HortTech. 7(1):23-29.

Tyree, M.T. 1988. A dynamic model for water flow in a single tree: evidence that models must account for hydraulic architecture. Tree Physiol. 4:195-217.

Will, R.E., G.A. Barron, E.C. Burkes, B. Shiver, and R.O. Teskey. 2001. Relationship between intercepted radiation, net photosynthesis, respiration, and stem volume growth of *Pinus taeda* and *Pinus elliottii* stands of different densities. For. Ecol. Manage. 154:155–163.

Young, R.E. and G.R. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. J. Environ. Hort. 14:170-176.

# CHAPTER THREE

# GROWTH AND PHYSIOLOGICAL RESPONSE OF CONIFERS TO FERTILIZER AND MEDIA IN POT-IN-POT PRODUCTION FOR NORTHERN CLIMATES

**Abstract**

Container production of landscape conifers, including pot-in-pot (PIP) production, is increasing relative to field production in the northern US. Since much of the research on PIP has been performed in the southern US, this study focused on characterizing the growth and physiological response of PIP-grown conifers to fertilizer and media in order to improve production for growers in northern climates. In May 2006, we potted ninety seedlings (2+2 or plug+2) each of *Abies fraseri*, *Picea glauca* var. *densata*, *P. pungens glauca*, and *Pinus strobus*. Substrate consisted of pine bark (B) and peat moss (PM) in ratios of 90:10, 80:20 or 70:30 (vB:vPM). Trees were top-dressed with fertilizer at rates of 2, 4, and 8 g fertilizer $L^{-1}$ container. Growth response to media varied by species; however, all species grew as well or better in the 80:20 mix than in the other mixes. In response to fertilizer addition, adding 4 or 8 $g{\cdot}L^{-1}$ increased height growth compared to 2 g; increasing the rate from 4 to 8 g, however, did not increase height growth. Stem volume growth responded positively to fertilizer addition with maximum growth occurring between 4 and 8 $g{\cdot}L^{-1}$. Foliar nitrogen increased with each fertilizer addition even though height growth did not increase beyond 4 g, indicating possible luxury consumption. Furthermore, net photosynthesis rates were not affected by fertilizer rate, possibly due to increased water stress as a result of greater total leaf area (TLA) per tree. Recommendations to growers include using the 80% bark:20% peat moss media combination and fertilizing at 4 $g{\cdot}L^{-1}$. Chlorophyll fluorescence ($F_v/F_m$) was unrelated to $A_{max}$, and was negatively related to foliar N concentration during the second season.

## Introduction

Traditionally, landscape conifers have been field-grown and sold balled and burlapped (B&B). Soil loss due to harvesting field-grown trees can be nearly 100 tons per acre for a 5-year rotation and harvest can only take place in the spring and fall (Pollock and Mathers, 2002). If diseases such as phytophthora, especially common in firs, become established in soils, they can result in extensive mortality and in some cases have caused nurseries to be abandoned (Kuhlman et al., 1989). Moreover, consumer preference of container material has been steadily increasing (Halcomb and Fare, 1995). Container production has been increasing relative to B&B and now accounts for nearly 30% of the coniferous evergreen sales in the upper Midwest (NASS, 2007).

Pot-in-pot (PIP) production is an increasingly popular component of the overall container production trend. Since PIP plants are grown in containers, they have the same benefits as conventional above-ground container (AGC) plants, such as being lightweight, easy to harvest, and having root systems that are not disturbed by digging and transplanting (Ruter, 1997). However, unlike AGC production, the PIP containers are placed into socket pots which are sunk in the ground providing stability and protection of the root zone from extreme air temperatures. In southern nurseries, PIP production results in moderated root zone temperatures, especially during the hot summer months, and improves growth compared to field or AGC produced plants (Roberts, 1993; Ruter 1993, 1995, 1998, 1999). The system has also been adopted as a method of providing winter protection in northern climates (Neal, 2004).

Pot-in-Pot is also suitable for developing niche markets such as table-top and living Christmas trees. The upper Midwestern states account for up 22% of the gross sales of Christmas trees and Michigan ranks third nationally in total production behind Oregon and North Carolina (NASS, 2007) making it an important commodity. However, sales of real trees have been decreasing while sales of artificial trees have been increasing (NCTA, 2006). Living Christmas trees appeal to consumers who would otherwise choose artificial trees due to environmental concerns (Genovese, 2007). Other consumers desire a second or third Christmas tree for their home (Behe et al., 2005). "The pot-in-pot Christmas tree successfully addresses the issue of weight, handling, survivability, monetary value, and environmental stewardship" (Genovese, 2007). Some growers have found that Christmas trees as large as six feet tall can be grown in 37.85-L (10-gallon) pots using a lightweight media and still be manageable for a consumer to take into their home (Genovese, 2007). Small, dense trees such as Black Hills or Serbian spruce grown in a one gallon container could be placed on a table-top and decorated and are desirable options for a small apartment or as an additional tree (Behe et al., 2005). A study at Michigan State University showed that pot-in-pot trees taken inside for up to 20 days during the holiday season perform better in the landscape after transplanting compared to field-dug trees (Nzokou et al., 2007).

Landscape conifer growers converting from field production to PIP face several key challenges. Among these are selection of appropriate container media and nutrition management. The growing conditions in PIP systems are not the same as either field or AGC production. Lightweight organic media is used rather than field soil and root zone temperatures are more stable than in AGC plants (Young and Bachman, 1996). This affects the rate of water and nutrient uptake by the plants (Ruter, 1998). Fertilization regimes are often based on visual ratings (Parent et al., 2005) which can miss symptoms of 'hidden hunger' or 'luxury consumption' (Landis and van Steenis, 2004). Regular foliar analyses are recommended for determining nutrition regimes, however they can be costly and time consuming (Ritchie, 2006). A desirable alternative is to correlate foliar nutrition levels with indirect yet simple tests, such as SPAD or chlorophyll fluorescence, that growers can take with portable devices. SPAD 502 readings have been shown to be highly correlated with N and P levels in conifers (Parent et al., 2005). Chlorophyll fluorescence is indicative of plant stress, but it is not specific to any particular nutrient deficiency (Ritchie, 2006; Oren et al., 1993).

The goal of this project was to develop fertilizer and media recommendations to optimize growth of containerized conifers, reduce potential environmental impacts, and maximize profits for growers using the PIP production system. Specific objectives were to: 1) understand the physiological response of conifers to increasing fertilizer levels, 2) determine the effects of fertilizer addition on growth and nitrate leaching levels, 3) determine which of three media combinations resulted in the most growth, and 4) correlate foliar nutrition levels with chlorophyll fluorescent values, since the SPAD meter was not able to provide measurements for the conifer needles.

## Materials and Methods

### *Site Description*

This experiment was conducted at the Michigan State University (MSU) Horticulture Teaching and Research Center, Lansing, MI. The soil was a loamy sand (83.1% sand, 8.7% silt, 9.3% clay), which provided adequate drainage of the containers in the PIP system. In order to install socket pots for the PIP system, holes were made with a 40-cm diameter auger, spaced approximately 1 m on-center. We then placed landscape cloth over the entire plot, secured it with standard landscape staples, cut an 'x' in the cloth above each hole, and placed the socket pots (GL1200, Nursery Supplies, Inc., Chambersburg, PA) so the rims were approximately 2.5 cm above the surface of the ground. The mean daily temperature during the growing season (June through September) was 25 °C for 2006 and 26 °C for 2007; total precipitation during that time was 318 mm in 2006 and 295 mm in 2007 (MAWN, 2007). Average air temperature during the winter months (December 2006 through March 2007) was −1 °C with a minimum of −22 °C (MAWN, 2007).

*Plant Material*

In May 2006, 90 seedlings (2+2 or plug+2) each of *Abies fraseri* (Pursh) Poir., *Picea glauca* (Moench) Voss var. *densata* L.H. Bailey, *P. pungens* Engelm. var. *glauca* Regel, and *Pinus strobus* L. (average initial caliper and height given in table 2.2) were donated from local nurseries (Fairplains Nursery, Greenville, MI; Peterson's Riverview Nursery, Allegan, MI) and potted in 10.2-L (#3) containers (EG1200, Nursery Supplies, Inc., Chambersburg, PA).

*Container Media Treatment*

Seedlings were potted in one of three substrate mixes selected to provide a range of physical properties. Substrate consisted of composted pine bark (B) and Canadian peat moss (PM) in ratios (vB:vPM) of either 70%:30%, 80%:20%, and 90%:10% (Renewed Earth, Kalamazoo, MI). Thirty trees of each species were potted in each substrate mix.

*Fertilizer Treatment*

Controlled-release fertilizer (Osmocote® Plus 15-9-12, 8-9 month Northern release rate; The Scotts Co., Marysville, OH) was top-dressed in the spring of 2006 and

2007. Each tree received one of three rates corresponding to high (8 $g{\cdot}L^{-1}$) and low (4 $g{\cdot}L^{-1}$) manufacturer recommended rates, or one-half the low recommended rate (2 $g{\cdot}L^{-1}$), which was used to observe low nutrient responses.

*Irrigation*

Irrigation was initially applied using overhead sprinklers in order to promote establishment after transplanting. In mid-June 2006, micro-sprinkler spray stakes (TS-90, Chapin Watermatics Inc., Water Town, NY) were installed, one per pot, and set to the medium level, which applied approximately 0.35 $L{\cdot}min^{-1}$. Trees were then irrigated twice per day using an automated valve (8014 DuraLife, L.R. Nelson Corp., Peoria, IL) at 7:00 AM and 5:30 PM, for 5 min each cycle, totaling approximately 3.5 L of water applied daily per tree. Trees were irrigated from early May to mid-November of both 2006 and 2007.

*Experimental Design*

The experimental design was a three-way factorial arrangement of species × fertilizer × media within a randomized complete block. Trees were blocked to account for possible variability as a result taking physiological measurements throughout a day. There were 10 blocks, each consisting of 4 rows, one for each species; each row had 9 trees, one for each of the fertilizer × media combinations.

*Growth*

We measured initial height and caliper on 18 May 2006. Height was measured from the rim of the pot to the tip of the leader (most vertical central shoot); caliper was measured in an east-west orientation, level with the rim of the pot. Measurements were also taken on 12 and 29 June. On 17 July, terminal leaders of *P. strobus* were measured to determine if growth had stopped for the year; all terminal shoots on the pines were then pruned to 25-30 cm (in proportion to the overall height of the tree) according to standard nursery practices. On 23 March 2007, we measured total height, leader height, and caliper on all trees to use as the final growth measurement for the 2006 growing season. Initial measurements for the 2007 season were taken on 11 June, and final measurements were taken on 7 November.

Height and caliper growth for each season was calculated by subtracting the initial measurements from the final measurements. Volume indices were calculated by multiplying height growth by the square of the caliper ($d^2h$) for each season.

Projected shoot weights were estimated from stem volume indices using regression equations developed from destructive harvests of a subsample of 15 seedlings for each speceis.

*Gas Exchange: Single-needle Conifers*

We measured photosynthetic gas exchange on *A. fraseri*, *P. glauca* var. *densata*, and *P. pungens glauca* with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) in July and August 2006 and May, June, July, and September 2007. On each date, measurements were taken between 9:00 AM and 5:00 PM; however, data collection typically spanned multiple days. A 0.25-L conifer chamber attachment (LI-6400-05, Li-Cor) was used to enclose a single shoot of the current season's growth on each; light saturated photosynthesis ($A_{max}$; $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$), conductance ($g_{wv}$), and transpiration (E; $mol \cdot m^{-2} \cdot s^{-1}$) were measured on shoots exposed to full sunlight, on days with photosynthetic photon flux density (PPFD) greater than 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In order to reduce variation due to temperature, the chamber temperature was set to the predicted high temperature for each day of measurement. Air flow through the chamber was 500

$mL \cdot min^{-1}$, with the reference $CO_2$ concentration slightly above ambient, at 400 μmol $CO_2 \cdot mol^{-1}$. Vapor pressure deficit was maintained at approximately 3 kPa. The shoots were tagged so subsequent measurements were taken on the same shoot throughout each year. We collected the tagged shoots at the end of each growing season and scanned them with a leaf area meter (LI-3000, Li-Cor) to determine projected shoot area for $A_{max}$. Intrinsic water use efficiency (WUE) was calculated by dividing $A_{max}$ by $g_{wv}$ for each measurement.

*Gas Exchange: Pines*

We used the same portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE) for the pines; however, since the shoots would not fit in the conifer chamber, a 3 × 2 cm leaf chamber with a red/blue LED light source (LI-6400-02B, Li-Cor) with quantum flux maintained at 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used to enclose a portion of the needles. Gas exchange rates, including light saturated photosynthesis ($A_{max}$; $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) and conductance ($g_{wv}$), were measured on needles of *P. strobus* in July and August 2006 and May, June, July, and September 2007. We placed two fascicles of needles from the current season (a total of 10 needles) lengthwise in the chamber, making sure the needles did not overlap. Air flow through the chamber was 500 $mL \cdot min^{-1}$, with the reference $CO_2$ concentration slightly above ambient, at 400 μmol $CO_2 \cdot mol^{-1}$. The temperature within the chamber was again set to the predicted high temperature for each day of measurement, and vapor pressure deficit was maintained at approximately 3 kPa. Intrinsic water use efficiency (WUE) was again calculated by dividing $A_{max}$ by $g_{wv}$ for each measurement.

Since the LI-COR 6400 estimates gas exchange rates based on the area of the sample, average needle surface area was determined for *P. strobus* needles. Using a dissecting microscope, we measured the radius on a subsample of needles. Total surface area per fascicle was calculated by assuming each needle represented one-fifth of a cylinder (Johnson, 1984); since two fascicles were used per $A_{max}$ measurement, this value was then multiplied by two.

*Chlorophyll Fluorescence*

A portable chlorophyll fluorescence meter (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Norfolk, England) was used to measure the ratio of variable fluorescence to maximum fluorescence ($F_v/F_m$) for individual needles from each tree. Plastic/foam clips provided by the manufacturer were clipped to each needle, and the needle was centered in the measurement window. The needles were dark-acclimated for a minimum of 15 min before readings were taken. Dates of measurement of $F_v/F_m$ coincided with measurements of $A_{max}$.

*Container Substrate Moisture*

Container substrate moisture levels were measured to a depth of 15 cm using a portable time domain reflectometry system (TDR; Trase 6050X1, Soilmoisture Equipment Corp., Santa Barbara, CA) on measurement dates corresponding to dates of $A_{max}$ measurements. Measurements were taken twice per pot, and the values were averaged.

*Foliar Analysis*

We collected approximately five shoots or 20 fascicles from each tree on 15 August 2006 and again on 12 October 2007 for foliar nutrient analyses. To facilitate sampling and analyses, foliar samples were combined across media type. The shoots and fascicles were placed in paper bags and oven-dried at 60 °C for 1 wk. After drying, we removed the needles from the stems, if necessary, and ground them in a standard coffee grinder until they passed through a 0.42 mm mesh sieve. The prepared samples were sent to a commercial laboratory (Waters Agricultural Laboratories, Inc.,Camilla, GA) for analysis. In 2006, samples from three of the 10 blocks were analyzed for full foliar nutrient content and the remaining blocks were analyzed only for nitrogen content. All samples collected in 2007 were analyzed for full foliar nutrient content. Results were examined for species and fertilizer effects.

*Tree Leaf Area*

Total shoot or needle weights were determined for a subsample of trees at the end of the 2007 growing season. One block of trees was selected, and each tree was destructively harvested. For the single-needle conifers (SNC), shoots were separated by year; 2007 growth was removed first by pruning all shoots beyond the 2006 terminal bud scar, and placing them in a paper bag. Next, 2006 growth was removed (as determined by the 2005 terminal bud scar), and placed in a second paper bag. Finally, the remaining shoots were removed and placed in a third paper bag. For the pines, needles were removed from the shoots and also separated by year using the same method as for the SNC. Shoots and needles were oven-dried at 60 °C for 1 wk and then weighed. We then performed a regression analysis between the shoot or needle weights for the sampled block and their respective volume indices to estimate total shoot or needle weights for every tree. Due to a significant species effect, separate regression equations were developed for each species.

*Nitrate-N, pH, and EC in leachate*

The PourThru extraction procedure (Bilderback, 2001) was used to collect leachate from a sub-sample of two species, *A. fraseri* and *P. strobus*, with fertilizer × media combinations of 20 g × 70:30, 20 g × 90:10, 40 g × 80:20, 80 g × 70:30, and 80 g × 90:10. Samples were collected approximately every 2 wks from June to September 2006 and June to August 2007. The selected trees were removed from their socket pots and placed on pallets to provide drainage for the leachate, which was collected into 20 mL vials and stored in a cooler at 2.5 °C. Electrical conductivity (EC; ExStik II EC500, Omni Controls Inc., Tampa, FL) and pH (Accumet® basic AB15 meter, Thermo Fisher Scientific Inc., Waltham, MA) were measured in the laboratory within a week after collection. Nitrate-N analysis was then conducted by the MSU Soil and Plant Nutrient Laboratory (SPNL) using flow-injection with cadmium reduction (Huffman and Barbarick, 1981).

*Statistical Analysis*

All variables were tested for normality using PROC UNIVARIATE and Levene's test. Height and caliper growth were normalized using a squareroot transformation. A log transformation was used to normalize stem volume growth; $NO_3$ and EC in leachate; pine $A_{max}$ and transpiration data; and transpiration and conductance for the single-needle conifers. Chlorophyll fluorescence was normalized by squaring the data.

PROC MIXED (SAS Inc., Cary, NC) was used to determine type 3 analyses of variance (ANOVA) for species, treatment, date, and interaction effects. Effects over time for gas exchange, nitrate-N, pH, EC, and $F_v/F_m$ data were analyzed using repeated measures within PROC MIXED. Correlations between height, stem caliper relative growth rate, volume, $A_{max}$, E, $F_v/F_m$, N, P, K, Mg, B, Mn, Cu, pH, and $NO_3$ were determined using PROC CORR. Pearson's correlation coefficients were determined between projected shoot weight and volume indices, seasonal volume growth and foliar N levels, and $F_v/F_m$ and foliar N levels.

*Considerations*

On 7 July 2006, Subdue Maxx (Syngenta, Inc., Greensboro, NC) was prepared by diluting 15 mL fungicide in 95 L water, and 1 L was applied to each *A. fraseri* to control a *Phytophthora* outbreak; 13 trees did not recover and were removed from the study. Media type did not have an effect on the occurrence of *Phytophthora*.

On 17 May 2007, a frost advisory was issued, so the trees were covered with frost fabric, which accidentally damaged some of the new growth.

## Results

### *Growth Responses*

The interaction fertilizer × media effects for growth were not significant, therefore the growth response to fertilizer is presented across all media types. Height growth was affected by fertilizer treatments in 2007 but not in 2006 (fig. 2.1). In the second season, addition of 4 or 8 $g \cdot L^{-1}$ container increased height growth compared to 2 g ($p<0.0001$) for all species (table 2.1). In 2006, stem caliper increased with the addition of 8 $g \cdot L^{-1}$ container. Caliper was more responsive than height to fertilizer treatments in 2006 (fig. 2.1). In 2007, caliper responded positively to fertilizer addition, with a maximum between 4 and 8 $g \cdot L^{-1}$. Stem caliper relative growth rate (RGR) was used to account for initial differences in tree size, particularly between species (table 2.2). The species × fertilizer × year interaction effect was significant ($p<0.01$) for caliper RGR (table 2.1). In

2006, the relative growth rate was greater at the higher fertilizer addition for all species. No clear trend was observed in 2007.

Media composition also affected ($p<0.01$) stem caliper. Since there were no interactions, it was possible to generalize the media response across all species and fertilizer levels. Caliper increase was greater for trees grown in the 80% pine bark: 20% peat moss or 70:30 media compared to the 90:10 media. Media did not affect caliper RGR.

Stem volume index ($d^2h$) was greater at the higher fertilizer level for the 2006 season; however, by the end of the 2007 season, there were no differences between the 4 and 8 g treatments (fig. 2.1). Stem volume index was also affected ($p<0.05$) by media type as well as the species × media interaction (table 2.1, fig. 2.2). With the exception of *P. strobus*, volume increase followed the same pattern as caliper increase, with the 80:20 and 70:30 media combinations having greater growth compared to the 90:10 combination (fig. 2.2). Overall, volume index was greatest for *P. strobus* while *P. glauca* var. *densata* and *A. fraseri* grew the least during the study.

Total shoot weights, estimated from volume indices, were generally greater at higher fertilizer rates (fig. 2.3). The overall trends each season were the same as for volume growth.

***Physiological Responses***

Due to differences in shoot morphology, gas exchange rates of the single-needle conifers (*Abies* and *Picea* species) are presented on projected shoot area basis, while *P. strobus* measurements are based on total needle area. Therefore, results for the gas exchange parameters will be discussed separately for the two groups.

*Photosynthesis: Single Needle Conifers*

Photosynthetic rates for the single-needle conifers varied between nearly 0 to around 20 ($\mu$mol $CO_2 \cdot m^{-2}s^{-1}$) throughout the growing seasons (data not shown). The fertilizer and media main effects did not influence $A_{max}$ when averaged over both years, however, the species × fertilizer × date interaction affected ($p<0.01$) rates (table 2.3). In 2006, photosynthetic rates were generally greater at higher fertilizer levels. During the 2007 season, however, $A_{max}$ was either not affected or was lower at higher fertilizer rates. When the data were averaged across species and fertilizer treatment, $A_{max}$ was greater at higher peat moss volumes during the first growing season. However, in 2007, there were no differences between rates for the varying media compositions. Species greatly affected ($p<0.0001$) $A_{max}$ (table 2.3); rates were nearly double for the *Picea* species compared to *A. fraseri*. Due to an outbreak of phytophthora, photosynthetic rates were only measured once for *A. fraseri* during the 2006 season.

Intrinsic water use efficiency (WUE; $A_{max}/g_{wv}$) varied in response to species and fertilizer effects (table 2.3). The species × date and fertilizer × date interactions were also significant ($p<0.01$). In 2006, WUE was lower with higher fertilizer additions for *A. fraseri*; in 2007, WUE was greater with higher fertilizer additions for all three species (fig. 2.4). When averaged across fertilizer levels, *A. fraseri* had higher WUE compared to the *Picea* species for both years.

*Photosynthesis: Pines*

Photosynthetic rates for *P. strobus* were not affected by media in 2006, but were affected ($p<0.01$) by fertilizer and date (table 2.4). $A_{max}$ was generally higher for the 4 and 8 $g \cdot L^{-1}$ treatments compared to the 2 $g \cdot L^{-1}$ treatment. Photosynthetic rates decreased from the beginning of July through August 2006 (data not shown). In 2007, $A_{max}$ was affected by date but not fertilization; rates increased from May to June and June to July, but decreased again by September. Fertilization affected ($p<0.05$) conductance in 2007 (table 2.4). Rates for both parameters were greatest for the 4 $g \cdot L^{-1}$ container treatment, when averaged across species and date.

Averaged across all four conifer species, photosynthetic rates were negatively correlated with height ($p<0.0001$) and volume ($p<0.01$) growth (table 2.5). Conversely, total stem caliper growth and caliper relative growth rate (RGR) were positively correlated with $A_{max}$ ($p<0.05$).

*Media Moisture*

In 2007, the addition of 4 or 8 $g \cdot L^{-1}$ reduced ($p<0.05$) media moisture content compared to the 2 $g \cdot L^{-1}$ treatment, suggesting increased moisture depletion associated with greater growth at the higher fertilizer levels. Media containing 30% peat moss had higher moisture content ($p<0.05$) than media with 10% peat moss (fig. 2.5).

***Foliar Nutrient Concentrations***

*Foliar analyses*

Concentrations of N in foliar samples varied ($p<0.0001$) by species (table A2.4). The two *Picea* species had greater N concentrations compared to *A. fraseri* and *P. strobus* over both years, and in 2007, *P. pungens glauca* had higher levels than *P. glauca* var. *densata* (table 2.6). Nitrogen concentration in needles increased with increased fertilizer addition for all species, however, differences between the 2 and 4 g per container treatments were more pronounced in 2006 than in 2007.

In 2006, foliar N levels of *A. fraseri* and *P. strobus* were below 1.5% for the 2 and 4 $g \cdot L^{-1}$ fertilizer treatments; both *Picea* species had foliar N levels below 1.5% for the 2 g treatment (table 2.6). Concentrations in 2007 samples were greater than 1.5% for all species and treatments.

Volume growth and foliar N concentrations typically had a positive relationship (fig. 2.6). Nitrogen was negatively correlated ($p<0.0001$) with P, K, and Mg (table 2.5), indicating possible uptake inhibition. Another interpretation is that the increased growth diluted the absolute content of the minerals in the leaf, thereby decreasing the concentration.

*Indirect Measurements: Chlorophyll fluorescence*

Species, fertilizer, and date all affected ($p<0.0001$) chlorophyll fluorescence ($F_v/F_m$) (table A2.5). In general, *A. fraseri* had the lowest $F_v/F_m$ values. When data were averaged across media combinations, $F_v/F_m$ increased with increasing fertilizer addition for *A. fraseri* and *P. strobus*. Fluorescence levels generally increased throughout the 2007 growing season, no consistent trends were observed in 2006. Chlorophyll fluorescence was unrelated to $A_{max}$ in both seasons. In 2006, $F_v/F_m$ was positively correlated ($p<0.0001$) with foliar N concentrations (fig. 2.7); however, during the second season, the relationship was negative ($p<0.01$); correlations were negative between $F_v/F_m$ and K ($p<0.01$) and Mg ($p<0.05$).

***PourThru Leachate Samples***

Since leachate was collected for a subsample of species and fertilizer × media combinations, not all species, fertilizer, and media comparisons were possible. However, practical conclusions can still be drawn from the available data.

Leachate $NO_3$ levels were affected ($p<0.0001$) by fertilizer level (table A2.6). Concentrations were consistently higher with the 8 $g \cdot L^{-1}$ container fertilizer treatment compared to the 2 or 4 g per container treatments (fig 2.8). The species × fertilizer × date interaction ($p<0.05$) reflected the rapid decline in $NO_3$ concentration for all treatments during late summer, eliminating the differences between treatments. Although $NO_3$ concentrations also varied ($p<0.05$) between the two species sampled, trends were not consistent across fertilizer level or date; on some occasions, $NO_3$ concentration was greater in leachate from *A. fraseri*, while other times the concentrations were greater for *P. strobus*.

Overall, leachate $NO_3$ concentrations peaked in mid-July, before rapidly declining in late summer (fig. 2.8). In 2006, $NO_3$ concentrations for both *A. fraseri* and *P. strobus* generally increased with increasing fertilizer addition; for all treatments, concentrations remained below 50 mg $L^{-1}$. Concentrations in 2007 were generally higher than in 2006. During the second season, $NO_3$ concentration also increased with fertilizer addition; values for *A. fraseri* were greater than 50 mg $L^{-1}$ for the 8 g treatment (fig. 2.8). Ranges were similar for *P. strobus*.

The media main effect did not influence $NO_3$ concentration, however, the media × date interaction was significant ($p<0.01$) (table A2.6). When data were averaged across species, the 70:30 media combination typically resulted in the highest N-leaching during the first season, while in the second season, greater $NO_3$ concentrations were found in leachate from the 90:10 media (not shown).

Leachate pH levels were affected ($p<0.0001$) by fertilizer (table A2.6); however effects for species or for the species × fertilizer interaction were not significant, so conclusions can be generally applied. Initial pH, taken on 10 July 2006, was 6.31(±0.005SE) for *P. strobus*. It should be noted that the *A. fraseri* were not tested on this date since they were being treated for phytophthora; however, since the species effect was not significant, it is safe to assume that the initial pH would also have been 6.31. Throughout the rest of the 2006 season, pH ranged between 7.4 and 8.2 for all treatments. In 2007, the 8 g treatment had a lower pH than the 2 and 4 $g \cdot L^{-1}$ treatments (fig. 2.8); pH for the 2 and 4 g treatments ranged between 7.1 and 7.4, while the 8 g treatment had pH levels between 6.8 and 7.1.

Media did not affect pH levels, although both the species × media and species × media × date interactions were significant ($p<0.05$) (table A2.6). For samples taken on 24 July and 8 August 2006 for *A. fraseri*, pH levels were lower for the 80:20 mix than for the 90:10 mix, and still lower for the 70:30 mix (data not shown).

Electrical conductivity (EC) was affected ($p<0.0001$) by species and date, as well as the species × date and fertilizer × date interactions (table A2.6). Levels increased with increasing fertilizer addition, and across all dates, EC levels for *A. fraseri* were either similar to, or less than levels for *P. strobus*. The fertilizer × media × date interaction was also significant ($p<0.05$).

**Discussion**

The goal of this project was to develop recommendations for fertilizer applications and media type in order to optimize growth of conifers in pot-in-pot (PIP) production systems. Proper recommendations will help maximize profits for growers using the PIP production system as well as reduce potential environmental impacts. The results of this study were used to: 1) characterize growth responses to various fertilizer levels and media combinations; 2) better understand the physiology driving the growth responses; 3) correlate results of indirect measurements, specifically chlorophyll fluorescence, with foliar nutrient levels and overall tree health; and 4) develop media and nutrition recommendations for conifers produced in PIP systems in Northern climates.

***Growth Responses***

*Response to Fertilizer*

Height, caliper, and volume growth were less responsive to fertilizer treatments during the 2006 growing season compared to the 2007 season; caliper growth was more responsive to treatments in 2006 than height growth. This is not surprising considering that height growth typically occurs early in spring, and is largely influenced by bud formation during the previous season; in contrast, caliper growth continues throughout the season and is more affected by the current environmental conditions and cultural factors. During the second season, the trees were more reflective of the treatments applied in the study than in seedling production, and differences between treatments were more clearly expressed.

In this study, maximum growth in 2007 occurred with fertilizer additions between 4 and 8 $g \cdot L^{-1}$ container. This is consistent with conventional recommendations, which suggest adding 5.23 $g \cdot L^{-1}$ container (equivalent to 3 g N per 1 gallon container).

*Response to Media*

Media moisture was affected by media type. The 70% pine bark: 30% peat moss combination had consistently higher moisture levels compared to the 90:10 combination.

Media did not greatly affect growth in either season. Of the three combinations studied, the intermediate ratio (80% pine bark to 20 % peat moss) resulted in sufficient growth for all species. Therefore, growers using pine bark and peat moss substrates can potentially use a single mix for all taxa compared in this study, which is much more practical than having to mix special combinations for different species.

***Physiological Responses***

*Photosynthetic Response*

Photosynthetic rates of the single-needle conifers were affected by fertilization; however, higher $A_{max}$ was associated with lower fertilizer levels. Conversely, $A_{max}$ was positively correlated with foliar N concentration for both seasons. Total shoot area, as estimated by linear regression of volume indices, generally increased with increasing fertilizer addition. The greater shoot area results in increased transpirational water loss. Furthermore, the increased growth response to additional fertilizer resulted in greater water stress at the higher levels, thereby decreasing the tree's photosynthetic capacity. This is supported by the decrease in leaf conductance with increased fertilizer addition. Media moisture content, measured on the same dates as $A_{max}$, also decreased with increasing fertilizer addition.

In this study, fertilizer did not reduce photosynthetic rates for the white pine as it had for the single-needle conifers. Since the growth rates of the pines, when averaged across treatment, were much greater than the spruces or fir, it is likely that trees in the 2 $g \cdot L^{-1}$ container treatment were under as much water stress as the 4 or 8 g treatment, thereby negating any increased photosynthetic potential at the higher treatment levels.

*Foliar Nutrition*

Critical nutrient levels have been defined for various conifer species, particularly those common in Christmas tree production. In a study in New York, Slesak and Briggs (2007) found that critical foliar N concentrations ranged between approximately 1.4% and 1.8% for various species of *Abies*. Rothstein and Lisuzzo (2006) referred to a critical level of 1.5% for their study on *A. fraseri*. In this study, growth and foliar N concentrations were related in 2006, however, since growth continued to increase with increasing N concentration, critical levels could not be determined. This is probably because trees were still affected by nutrients applied during seedling production, and were not fully responding to the current treatments. Moreover, since the trees were still relatively small with low nutrient requirements, the fertilizer applications may not have been limiting even at the low rate during the first season.

In 2007, critical levels were defined as the foliar concentration of N above which growth rate slows compared to the initial increase. The critical level for *A. fraseri*, according the results of this study, was approximately 1.8%, which is close to the value reported by Slesak and Briggs (2007) and Rothstein and Lisuzzo (2006). Growth rate for *P. strobus* also leveled off around 1.8%. The *Picea* species had slightly higher critical levels: around 2.1% for *P. glauca* var. *densata*, and 2.4% for *P. pungens glauca*.

*Indirect Measurements*

Previous studies have shown potential for relating chlorophyll fluorescence ($F_v/F_m$) with foliar nutrient concentrations and gas exchange rates (Ritchie, 2006; Strand, 1996). In this study, $F_v/F_m$ was unrelated to $A_{max}$. Chlorophyll fluorescence ($F_v/F_m$) values were correlated with N, however during the second growing season, higher foliar N concentrations corresponded to lower $F_v/F_m$ values. Growers are cautioned to rely on such measurements without developing specific protocols for their crops and site conditions.

***Leaching***

Nitrate-N levels in leachate collected in 2006 were below the recommended 50 to 100 mg·L$^{-1}$ (Bilderback, 2001) for all treatments and both species. However, this did not negatively affect the growth rates during that season. In 2007, only the 8 g treatment for either species had $NO_3$ concentrations greater than 50 mg·L$^{-1}$ during the season even though growth rates between the 4 and 8 g treatments were not different.

It is unclear what caused the differences in $NO_3$ levels between 2006 and 2007. Differences in temperature between years may have affected the release rate of the polymer-coated fertilizer pellets. Considering the low year-end levels of $NO_3$ in leachate concentrations, it is unlikely that residual fertilizer from 2006 affected the 2007 levels.

The negative correlation between leachate pH levels and foliar Mn concentrations (table A2.7) is consistent with previous findings, which have shown that in substrates with high pH, Mn precipitates and becomes unavailable for uptake (Mathers et al., 2007). The similar relationship between high pH and low N concentrations could also be a result of negative interactions.

***Grower Recommendations***

Based on growth and physiological responses, conifers grown in PIP systems in northern climates should receive approximately between 4 and 8 g·L$^{-1}$ container.
Our results indicate that it is acceptable for growers to use one standard mix for the taxa in this study, with the recommended ratio being 80% pine bark to 20% peat moss.

When monitoring foliar N, levels should be at least 1.8% for *A. fraseri* and *P. strobus*, 2.1% for *P. glauca* var. *densata*, and 2.4% for *P. pungens glauca* for foliar samples collected late in summer.

Table 3.1: Summary analysis of variance for caliper, height, and volume growth ($d^2h$), and caliper relative growth rate (cal RGR) of *Abies fraseri*, *Picea glauca* var. *densata*, *Picea pungens glauca*, and *Pinus strobus* grown in a PIP system under three fertilizer rates and three media combinations.

| Source of variation | | F-values | | | |
|---|---|---|---|---|---|
| | d.f. | caliper | height | $d^2h$ | cal RGR |
| Between subjects | | | | | |
| Species (Spp) | 3 | 10.90*** | 47.22*** | 10.45*** | 3.81* |
| Fertilizer (F) | 2 | 91.77*** | 68.47*** | 115.40*** | 41.78*** |
| Spp × F | 6 | 0.93 | 1.12 | 1.02 | 3.73** |
| Media (M) | 2 | 5.92** | 2.41 | 5.82** | 2.09 |
| Spp × M | 6 | 1.96 | 2.34* | 2.17* | 1.78 |
| F × M | 4 | 0.80 | 0.53 | 0.58 | 0.46 |
| Spp × F × M | 12 | 1.56 | 2.19* | 1.28 | 1.17 |
| Block (Blk) | 9 | 1.82 | 1.40 | 1.95 | 1.86 |
| Blk × Spp | 27 | 0.87 | 1.99* | 1.05 | 1.17 |
| Blk × Spp × F | 72 | 1.07 | 1.01 | 1.07 | 0.76 |
| Within subjects | | | | | |
| Year (Yr) | 1 | 1153.40*** | 20.83*** | 654.59*** | 299.80*** |
| Spp × Yr | 3 | 8.41*** | 7.62*** | 7.20** | 7.43*** |
| F × Yr | 2 | 7.99** | 24.77*** | 5.33** | 14.98*** |
| Spp × F × Yr | 6 | 5.10*** | 0.64 | 4.75** | 4.83** |
| M × Yr | 2 | 1.62 | 2.81 | 0.95 | 3.35* |
| Spp × M × Yr | 6 | 2.24* | 3.25** | 1.18 | 1.30 |
| F × M × Yr | 4 | 2.77* | 1.10 | 4.04** | 0.95 |
| Spp × F × M × Yr | 12 | 0.87 | 0.59 | 0.97 | 0.46 |
| Blk × Spp × F × M | 202 | 1.05 | 0.78 | 0.96 | 0.92 |

*, column means significantly different at $p<0.05$; **, column means significantly different at $p<0.01$; ***, column means significantly different at $p<0.0001$

Table 3.2: Means (±SE) of initial caliper and height, taken 18 May 2006, for four conifer species grown in a PIP production system under three fertilizer rates and three media combinations.

| Species | Caliper (mm) | Height (cm) |
|---|---|---|
| *Abies fraseri* | 9.43(± 0.18) | 30.90(± 0.68) |
| *Picea glauca* var. *densata* | 8.83(± 0.18) | 23.97(± 0.52) |
| *Picea pungens glauca* | 9.57(± 0.21) | 27.20(± 0.64) |
| *Pinus strobus* | 9.74(± 0.27) | 36.99(± 1.11) |

Table 3.3: Summary analysis of variance for photosynthesis ($A_{max}$), conductance ($g_{wv}$), and water use efficiency (WUE; $A_{max}/g_{wv}$) for single-needle conifers: *Picea glauca* var. *densata*, *Picea pungens glauca*, and *Abies fraseri* grown in a PIP production system under three fertilizer rates and three media combinations.

| Source of variation | 2006 F-values | | | | 2007 F-values | | | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | $A_{max}$ | $g_{wv}$ | WUE | d.f. | $A_{max}$ | $g_{wv}$ | WUE |
| Between subjects | | | | | | | | |
| Species (Spp) | 2 | 98.67*** | 97.33*** | 17.91*** | 2 | 89.07*** | 121.48*** | 14.96** |
| Fertilizer (F) | 2 | 54.45*** | 29.38*** | 0.19 | 2 | 14.19*** | 20.10*** | 18.55*** |
| Spp × F | 4 | 4.07** | 3.99** | 0.18 | 4 | 2.43 | 0.73 | 0.84 |
| Media (M) | 2 | 8.29** | 5.69** | 0.12 | 2 | 0.20 | 0.98 | 1.92 |
| SPP × M | 4 | 1.01 | 1.29 | 1.34 | 4 | 0.47 | 0.38 | 0.82 |
| F × M | 4 | 1.61 | 2.17 | 1.04 | 4 | 2.22 | 2.11 | 1.05 |
| Spp × F × M | 8 | 0.72 | 1.72 | 0.57 | 8 | 0.48 | 0.76 | 2.05* |
| Block (Blk) | 9 | 1.16 | 3.25* | 7.47** | 9 | 4.04** | 7.90*** | 3.43* |
| Blk × Spp | 18 | 1.33 | 1.97* | 3.79*** | 18 | 1.49 | 0.95 | 2.11* |
| Blk × Spp × F | 54 | 1.08 | 1.28 | 1.02 | 54 | 1.26 | 1.66** | 1.73** |
| Within subjects | | | | | | | | |
| Date (D) | 1 | 75.33*** | 10.45** | 67.74*** | 3 | 8.67*** | 0.39 | 7.29*** |
| Spp × D | 1 | 2.25 | 4.21* | 0.15 | 5 | 6.24*** | 12.82*** | 3.59** |
| F × D | 2 | 4.64* | 4.65* | 2.01 | 6 | 2.70* | 4.99*** | 1.38 |
| Spp × F × D | 2 | 0.08 | 0.49 | 0.88 | 10 | 1.43 | 1.60 | 0.46 |
| M × D | 2 | 0.04 | 1.41 | 1.88 | 6 | 1.00 | 1.32 | 0.45 |
| Spp × M × D | 2 | 0.20 | 1.03 | 0.31 | 10 | 1.13 | 1.24 | 0.42 |
| F × M × D | 4 | 1.58 | 2.58 | 1.35 | 12 | 0.51 | 0.80 | 0.40 |
| Spp × F × M × D | 4 | 0.59 | 0.54 | 0.11 | 20 | 0.88 | 1.25 | 0.66 |
| Blk × Spp × F × M | 147 | 1.36* | 1.26 | 0.75 | 149 | 1.26* | 1.84*** | 0.75 |

Table 3.4: Summary analysis of variance for photosynthesis ($A_{max}$), conductance ($g_{wv}$), and water use efficiency (WUE; $A_{max}/g_{wv}$) for *Pinus strobus* grown in a PIP production system under three fertilizer levels and three media combinations†

| Source of variation | 2006 F-values | | | | 2007 F-values | | | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | $A_{max}$ | $g_{wv}$ | WUE | d.f. | $A_{max}$ | $g_{wv}$ | WUE |
| Between subjects | | | | | | | | |
| Fertilizer (F) | 2 | 5.93** | 0.62 | 5.11* | 2 | 1.85 | 7.95** | 28.87*** |
| Media (M) | 2 | 2.33 | 1.54 | 0.88 | 2 | 1.00 | 1.64 | 2.23 |
| F × M | 4 | 0.54 | 0.96 | 1.53 | 4 | 2.18 | 1.27 | 0.23 |
| Block (Blk) | 9 | 9.05*** | 8.12*** | 12.75*** | 9 | 5.59** | 3.67** | 2.79* |
| Blk × F | 18 | 1.03 | 1.10 | 0.76 | 18 | 1.51 | 0.82 | 0.70 |
| Within subjects | | | | | | | | |
| Date (D) | 1 | 217.23*** | 11.04** | 75.89*** | 2 | 121.73*** | 181.56 | 344.43*** |
| F × D | 2 | 0.14 | 0.58 | 0.67 | 4 | 1.05 | 3.00 | 4.90** |
| M × D | 2 | 1.16 | 0.36 | 0.03 | 4 | 0.38 | 0.34 | 0.26 |
| F × M × D | 4 | 0.39 | 0.84 | 0.68 | 8 | 0.33 | 0.41 | 0.31 |
| Blk × F × M | 54 | 0.47 | 0.39 | 0.52 | 54 | 0.59 | 1.19 | 0.80 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

†Measurement taken on 10 July 2007 deleted due to instrumental error.

Table 3.5. Means for foliar N concentrations (in %N) for samples collected from *Abies fraseri*, *Picea glauca* var. *densata*, *Picea pungens glauca*, and *Pinus strobus* grown in a PIP production system under three fertilizer levels and three media combinations. Samples were combined by media type. Samples collected 15 August 2006 and 10 October 2007.

| Treatment | 2006 | | | | 2007 | | | |
|---|---|---|---|---|---|---|---|---|
| | *A. fraseri* | *P. glauca* | *P. pungens* | *P. strobus* | *A. fraseri* | *P. glauca* | *P. pungens* | *P. strobus* |
| 20 g | 0.92a | 1.41a | 1.22a | 1.05a | 1.60a | 1.98a | 2.18a | 1.73a |
| 40 g | 1.38b | 2.09b | 2.05b | 1.43b | 1.86b | 2.12a | 2.43b | 1.78a |
| 80 g | 1.83c | 2.38c | 2.59c | 1.73c | 2.19c | 2.38b | 2.94c | 1.96b |

*column means followed by different letters are significantly different ($p<0.05$).

Table 3.6. Pearson's correlation values for total height growth, caliper relative growth rate (RGR), total volume growth (vol), photosynthetic rate ($A_{max}$), conductance ($g_{wv}$), chlorophyll fluorescence ($F_v/F_m$), N, Mn, pH, and nitrate-N ($NO_3$) in pour-thru leachate for conifers grown in a PIP production system under three fertilizer levels and three media combinations.

| | RGR | vol | $A_{max}$ | $g_{wv}$ | $F_v/F_m$ | N | Mn | pH | $NO_3$ |
|---|---|---|---|---|---|---|---|---|---|
| height | 0.15*** | 0.58*** | -0.29*** | -0.34*** | 0.01 | -0.02 | 0.18*** | -0.10* | 0.05 |
| RGR | | 0.35*** | 0.05* | 0.02 | 0.18*** | 0.41*** | 0.24*** | -0.39*** | 0.25*** |
| vol | | | -0.06** | -0.14*** | 0.01 | 0.21*** | 0.16*** | -0.34*** | 0.32*** |
| $A_{max}$ | | | | 0.69*** | 0.09*** | 0.32*** | 0.16*** | -0.12** | 0.06 |
| $g_{wv}$ | | | | | 0.06** | 0.36*** | 0.21*** | -0.02 | 0.02 |
| $F_v/F_m$ | | | | | | 0.06** | 0.07** | 0.03 | -0.07 |
| N | | | | | | | 0.59*** | -0.45*** | 0.51*** |
| Mn | | | | | | | | -0.23*** | 0.54*** |
| pH | | | | | | | | | -0.43*** |

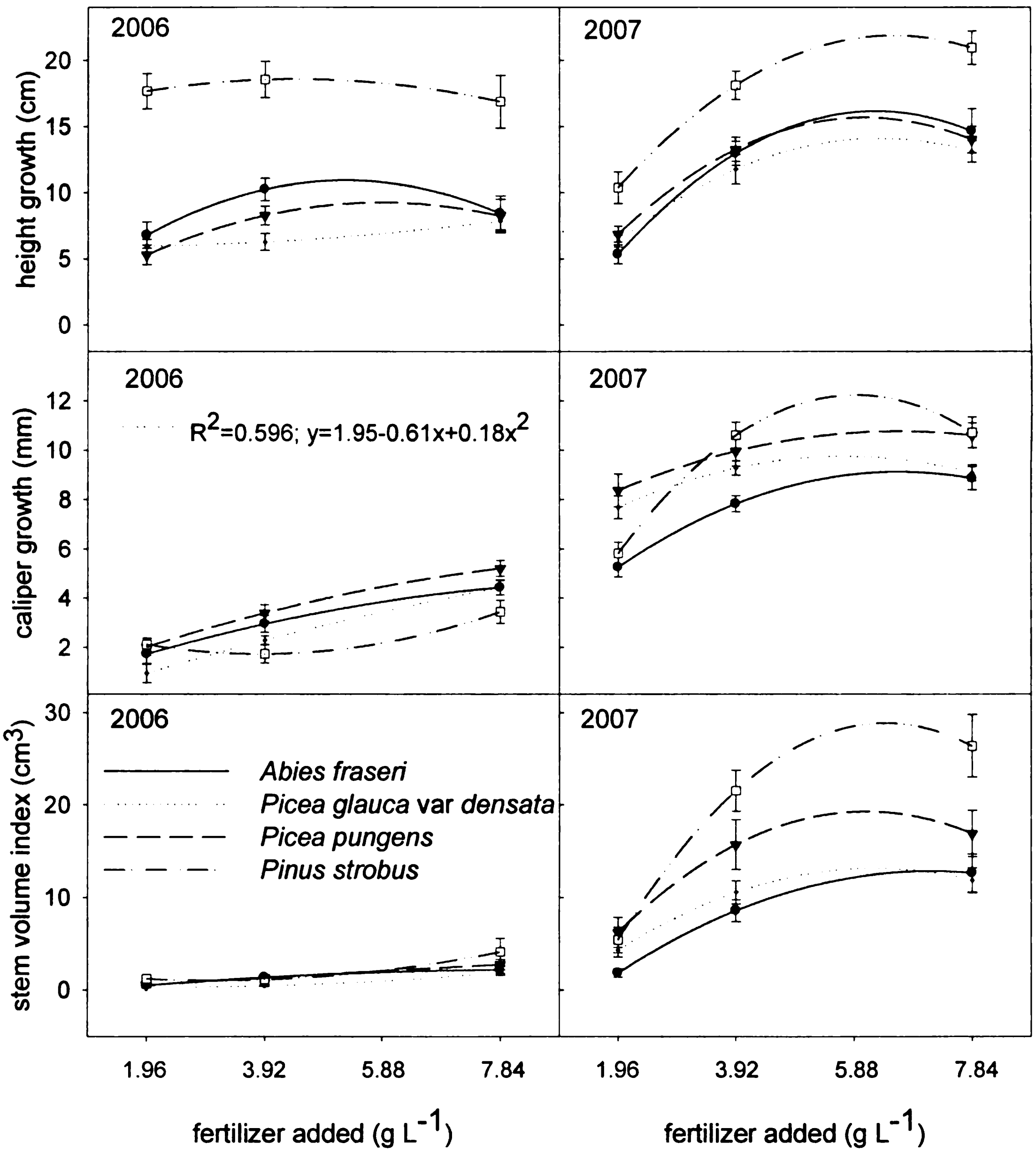



Figure 3.1. Height, caliper, and volume growth (± SE, comparison within species) in 2006 and 2007 for four conifer species grown in a pot-in-pot production system under three fertilizer levels. Equations with $R^2$ values <0.500 not shown.

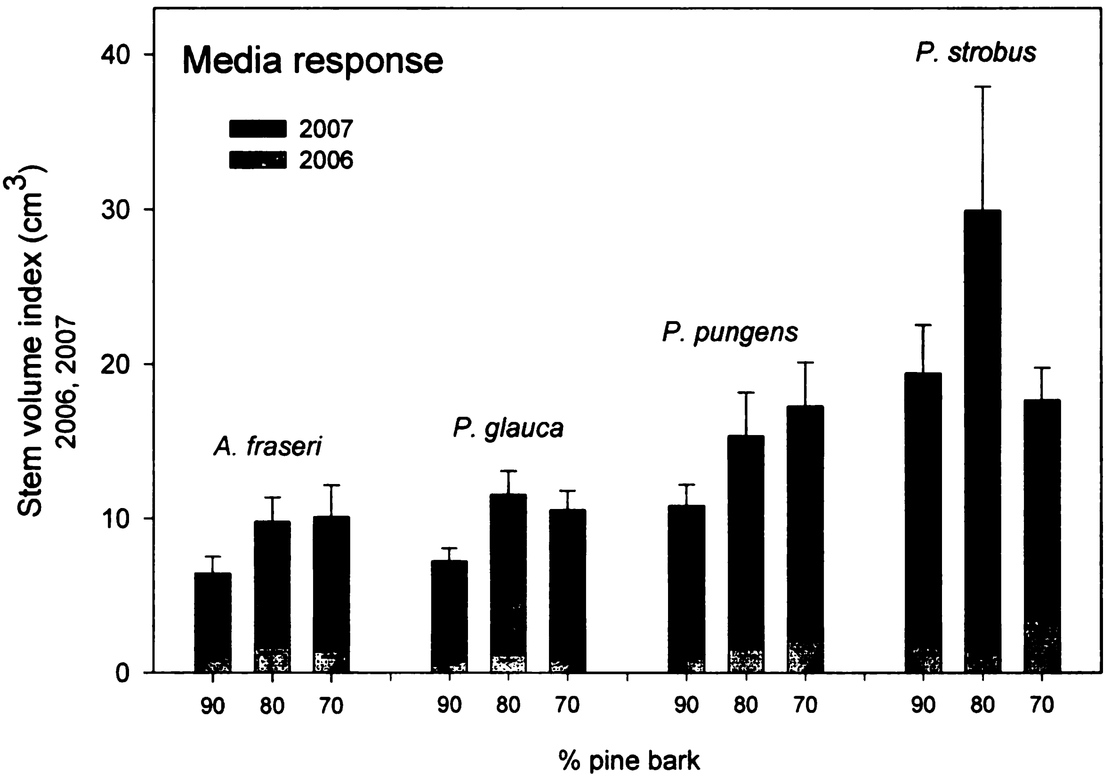



Figure 3.2. Stem volume index (cm$^3$) (± SE) to three different media combinations (v:v; 90% pine bark: 10% peat moss, 80:20, 70:30) for *Abies fraseri*, *Picea glauca* var. *densata*, *Picea pungens*, and *Pinus strobus* grown in a pot-in-pot production system; data averaged across three fertilizer levels.

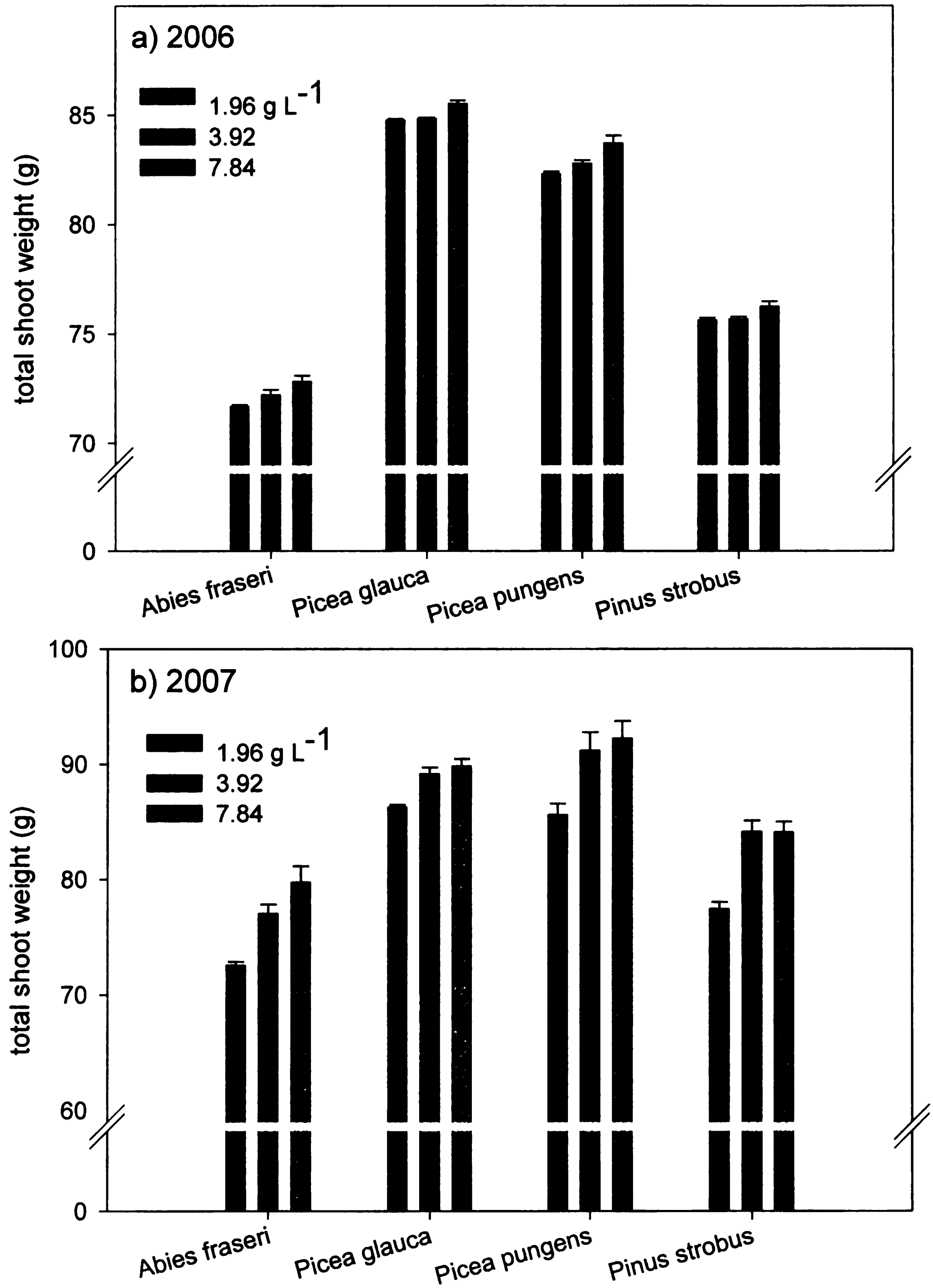



Figure 3.3. Total shoot weights (g; calculated from volume indices using linear regression) (± SE) for *Abies fraseri*, *Picea glauca* var. *densata*, *Picea pungens*, and *Pinus strobus* grown in a pot-in-pot production system under three fertilizer levels, averaged across three media combinations in a)2006 and b) 2007.

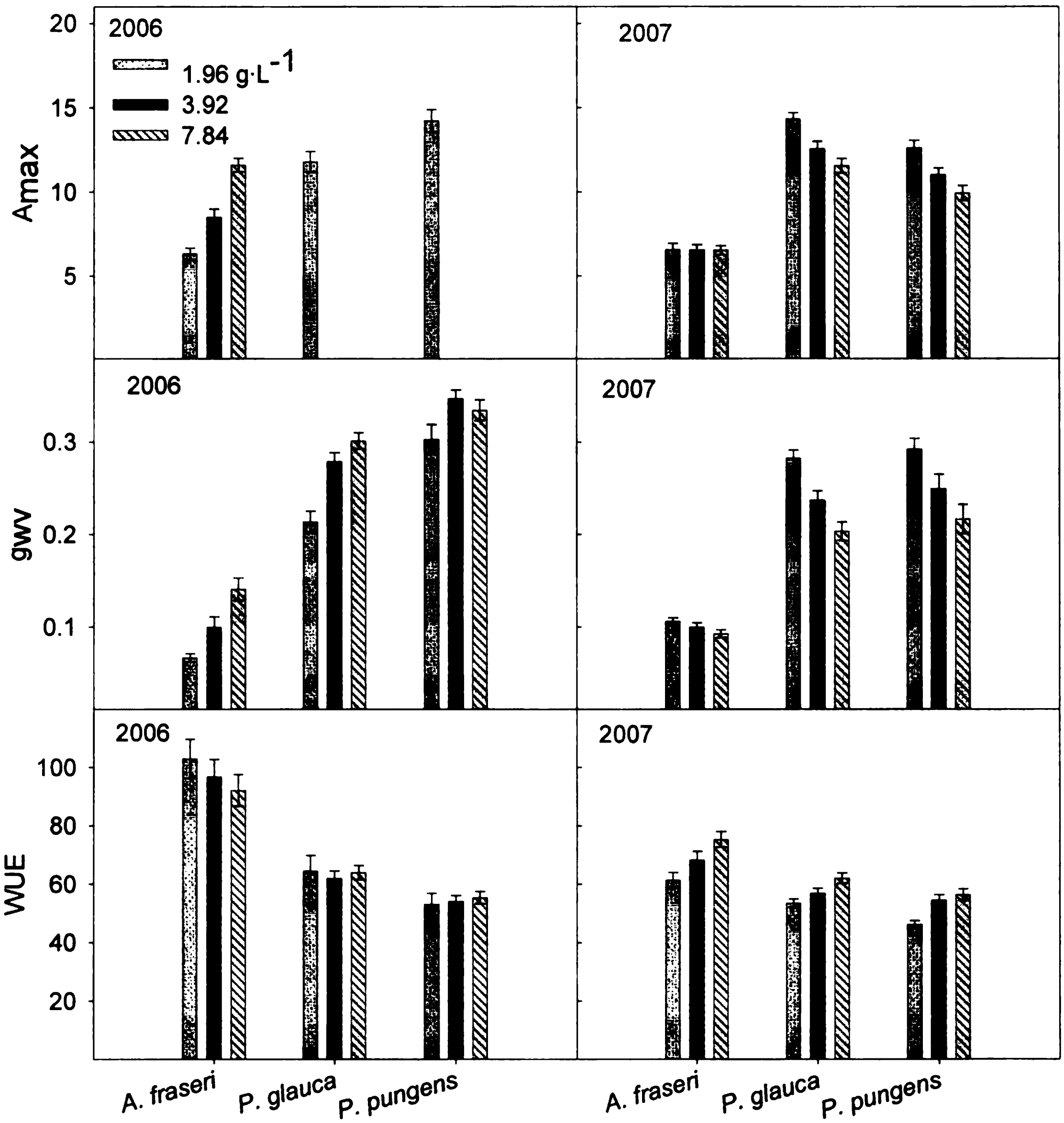



Figure 3.4. Photosynthetic rate ($A_{max}$; µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$), conductance ($g_{wv}$; mol $H_2O \cdot m^{-2} \cdot s^{-1}$), and water use efficiency (WUE; $A_{max}/g_{wv}$) (± SE) for *Abies fraseri*, *Picea glauca* var. *densata*, and *Picea pungens* grown in a pot-in-pot production system under three fertilizer levels, averaged across three media combinations.

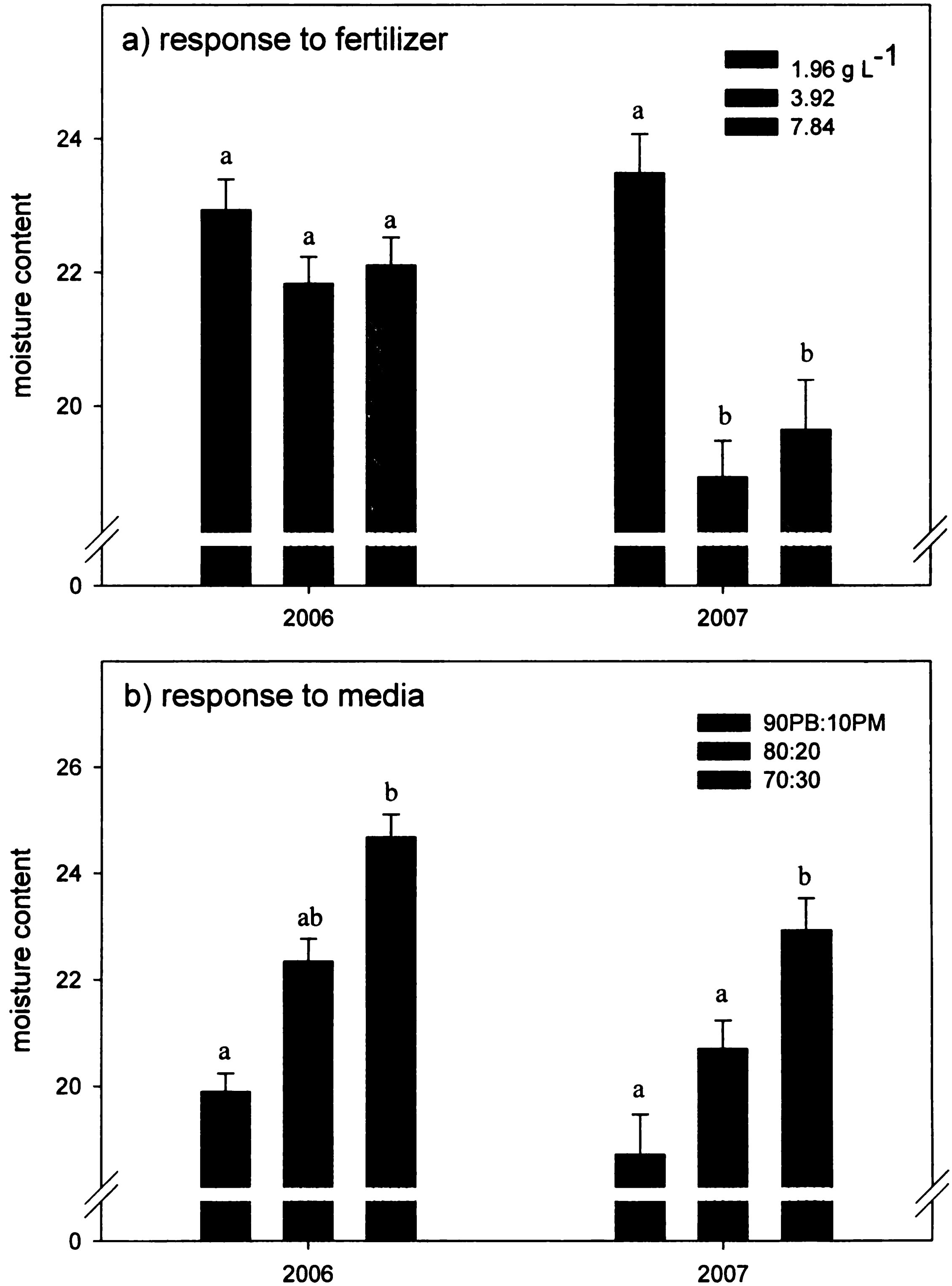



Figure 3.5. Media moisture content in response to a) fertilizer, and b) media in 2006 and 2007 for four conifer species grown in pot-in-pot production under three fertilizer levels and three media combinations. Different letters indicate means are significantly different using PROC lsmeans with Tukey's adjustment, $p \leq 0.05$.

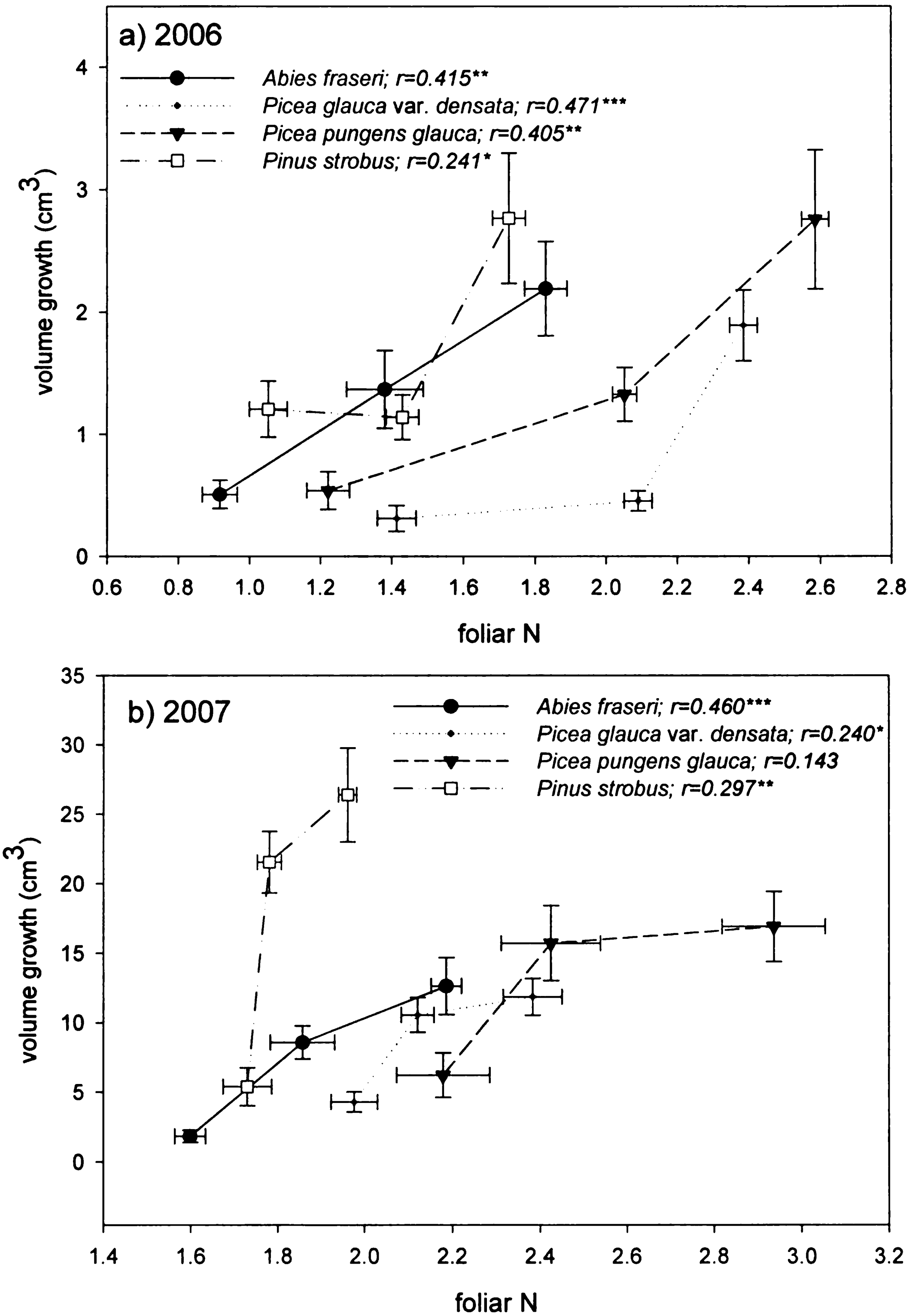



Figure 3.6. Pearson's correlation analysis between volume growth (± SE) and foliar N concentrations (%)(± SE) for four conifer species grown in a pot-in-pot production system under three fertilizer levels and three media combinations. Pearson correlation coefficients significant at * = p<0.05, ** = p<0.001, and *** = p<0.0001.

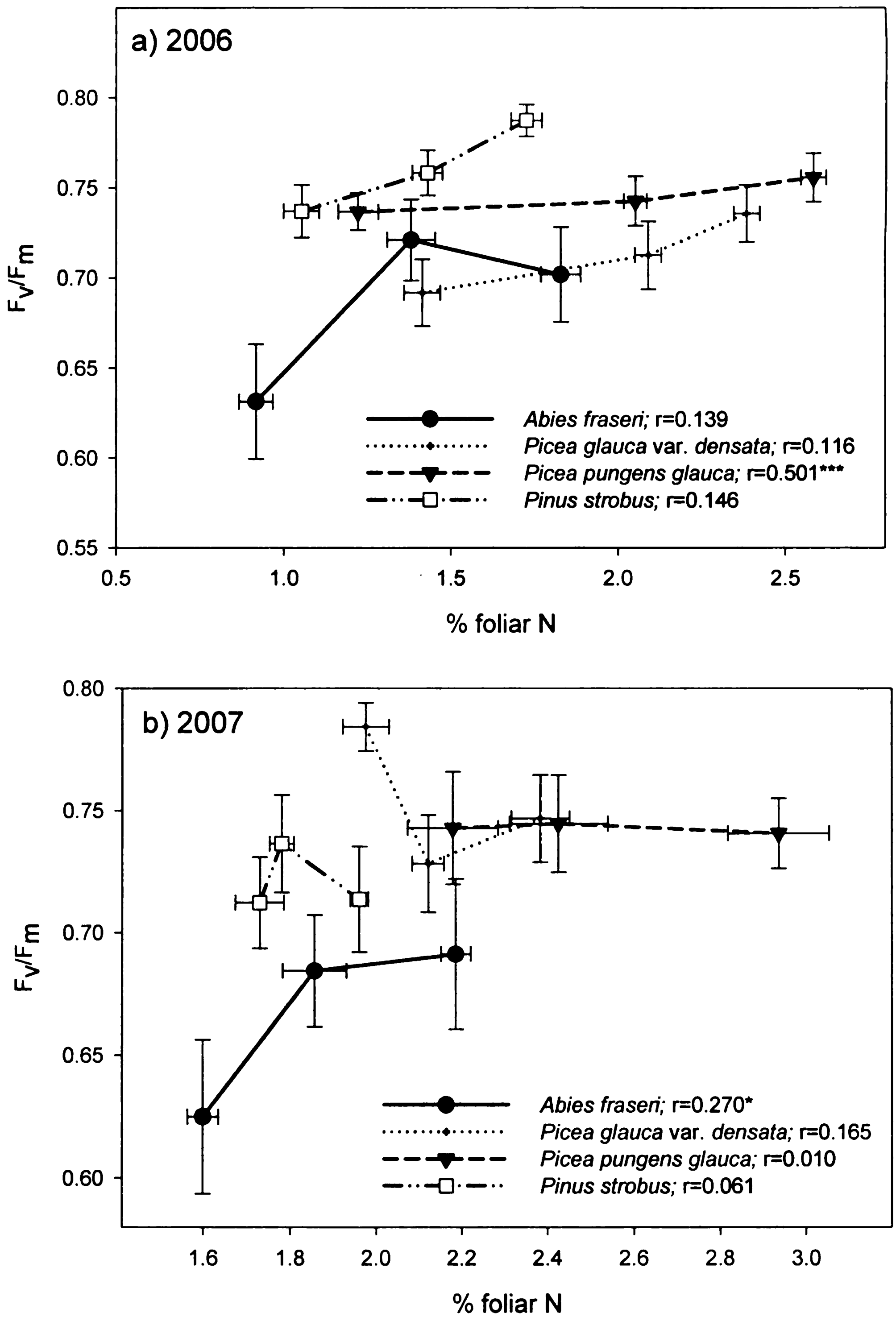



Figure 3.7. Pearson's correlation analysis between chlorophyll fluorescence ($F_v/F_m$) (± SE) and foliar N concentrations (%) (± SE) for a) 2006 and b) 2007, for four conifer species grown in a pot-in-pot production system under three fertilizer levels and three media combinations. Pearson correlation coefficients significant at * = p<0.05, ** = p<0.001, and *** = p<0.0001.

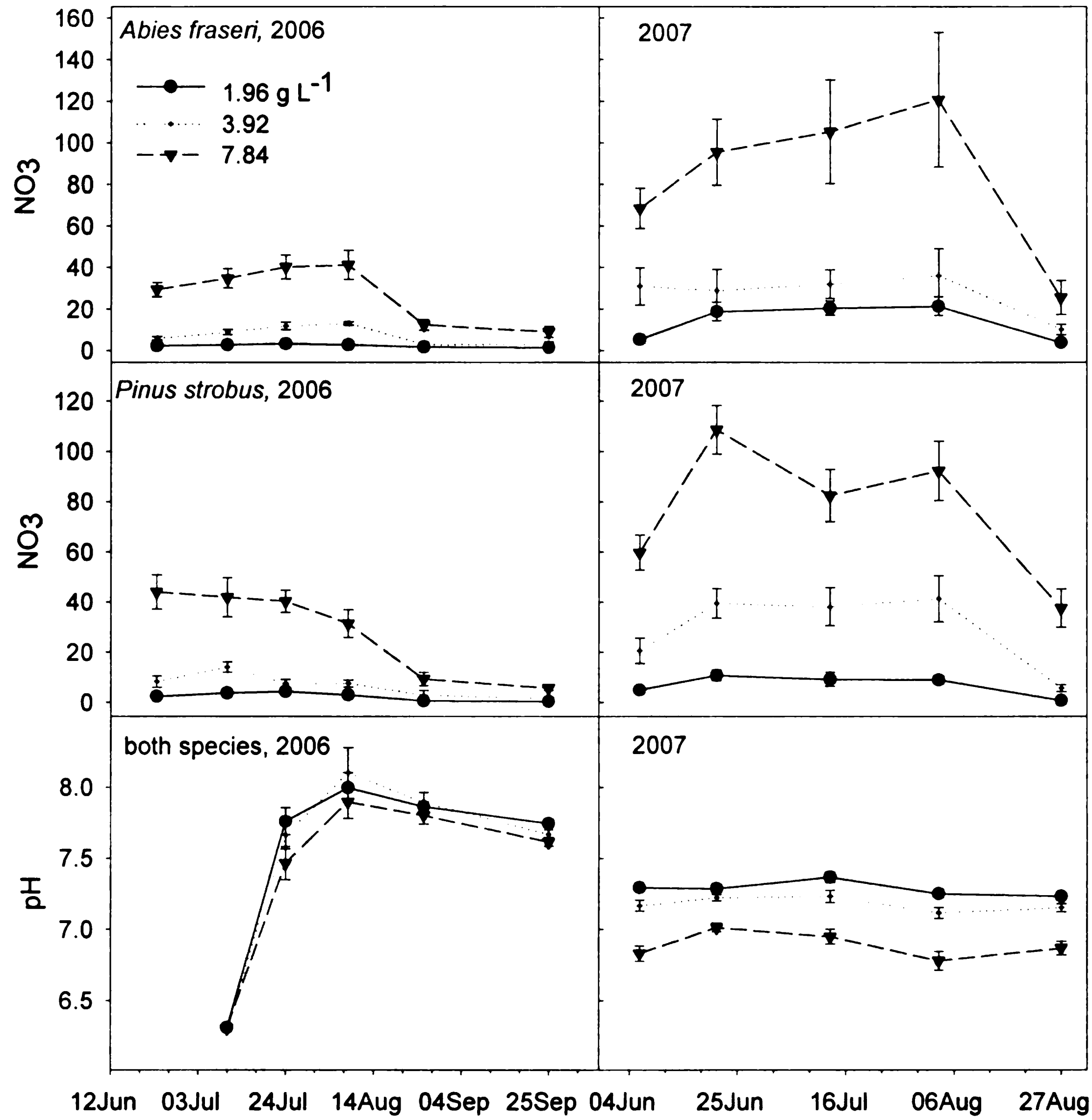



Figure 3.8. Nitrate-N concentrations (mg $L^{-1}$) and pH levels (± SE) for PourThru leachate samples for two conifer species grown in a pot-in-pot production system under three fertilizer levels, averaged across three media combinations.

Literature Cited


Behe, B.K., R.M. Walden, M.W. Duck, B.M. Cregg, and K.M. Kelley. 2005. Consumer preferences for tabletop Christmas trees. HortScience. 40(2):409-412.

Bilderback, T.E. 2001. Using the pourthru procedure for checking EC and pH for nursery crops. NC State University Horticulture Information Leaflet 450. 5 pp.

Genovese, F. 2007. Growing a new market for the Christmas tree industry: pot-in-pot Christmas tree production. Great Lakes Christmas Tree Journal 2(2):11, 29.

Halcomb, M.A. and D.C. Fare. 1995. A survey of the pot-in-pot growers in middle Tennessee. Proc. Southern Nursery Assoc. Res. Conf. 40:147-148.

Huffman, S.A. and K.A. Barbarick, 1981. Soil nitrate analysis by cadmium reduction. Communications in Soil Science and Plant Analysis. 12(1):79-89.

Kuhlman, E.G., L.F. Grand, and E.M. Hansen. 1989. Phytophthora root rot of conifers. In Forest Nursery Pests. USDA Forest Service, Agriculture Handbook No. 680, 184 pp.

Landis, T.D. and van Steenis, E. 2004. Macronutrients – nitrogen: part 2. Forest Nursery Notes. Winter 2004.

Mathers, H.M., S.B. Lowe, C. Scagal, D.K. Struve, and L.T. Case. 2007. Abiotic factors influencing root growth of woody nursery plants in containers. HortTech. 17(2):151-162.

MAWN. 2007. Michigan Automated Weather Network. 21 May 2008. http://www.agweather.geo.msu.edu/mawn/.

NASS. 2007. Nursery crops summary 2006. National Agricultural Statistics Service and United States Department of Agriculture. 18 January 2008. http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plants.

NCTA. 2006. Consumer survey results. National Christmas Tree Association. Found on internet at http://www.christmastree.org/statistics_consumer.cfm#legal.

Neal, C. 2004. Production Systems for Small Trees and Shrubs in New Hampshire. University of New Hampshire Cooperative Extension.

Nzokou, P., N.J. Gooch, and B.M. Cregg. 2007. Survivability of containerized live Christmas trees after indoor display. Great Lakes Christmas Tree Journal 2(4):4-9.

Oren, R., K.S. Werk, N. Buchmann, and R. Zimmermann. 1993. Chlorophyll-nutrient relationships identify nutritionally caused decline in *Picea abies* stands. Can J. For. Res. 23(6):1187-1195.

Parent, L.E., L. Khiari, and A. Pettigrew. 2005. Nitrogen diagnosis of Christmas tree needle greenness. Can. J. Plant Sci. 85: 939-947.

Pollock, C. and H.M. Mathers. 2002. Nursery production technique becoming a growing trend. Ohio State extension bulletin. 23 January 2002.

Ritchie, G.A. 2006. Chlorophyll Fluorescence: What Is It and What Do the Numbers Mean? USDA Forest Service Proceedings RMRS-P-43. 34-43.

Roberts, D.R. 1993. How Pot-in-Pot systems save time, money. Nursery Manager. 9(6):46-50.

Rothstein, D.E. and N.J. Lisuzzo. 2006. Optimal nutrition and diagnosis of *Abies fraseri* Christmas trees in Michigan. North. J. Applied For. 23(2):106-113.

Ruter, J.M. 1999. Production system influences growth of 'Kanzan' cherry and 'Chanticleer' pear. Proc. Southern Nursery Assoc. Res. Conf. 44:50-52.

Ruter, J.M. 1998. Fertilizer rate and pot-in-pot production increase growth of Heritage river birch. J. Environ. Hort. 16(3):135-138.

Ruter, J. M., 1997. The practicality of pot-in-pot. American Nurseryman. 185 (1):32-37.

Ruter, J.M. 1995. Growth of southern magnolia in pot-in-pot and above-ground production systems. Proc. Southern Nursery Assoc. Res. Conf. 40:138-139.

Ruter, J.M. 1993. Growth of three species produced in a pot-in-pot production system. Proc. Southern Nursery Assoc. Res. Conf. 38:100-102.

Slesak, R.A. and R.D. Briggs. 2007. Christmas tree response to N fertilization and the development of critical foliar N levels in New York. North. J. Appl. For. 24(3):209-217.

Young, R.E. and G.R. Bachman. 1996. Temperature distribution in large, pot-in-pot nursery containers. J. Environ. Hort. 14:170-176.

# CHAPTER FOUR

# COMBINED SUMMARY AND CONCLUSIONS

## Significance of the Study

Previous research on PIP production has focused mainly on growth responses; which generally indicate that the PIP system is well-suited for production of containerized trees and shrubs (Mathers, 2003; Neal, 2004; Ruter, 1998, 1995, 1993). This study was designed to go one step further and examine the physiological factors affecting growth, and how they are specifically affected by the PIP system. We measured growth responses, including height and caliper, as well as physiological responses, such as gas exchange and water use efficiency. By determining which physiological factors are affected by fertilization, we can then determine the mechanisms that drive growth. Our findings will assist nursery operators in designing management plans to maximize production efficiency while reducing potential environmental effects.

## Main results and ramifications

### *Deciduous Shade Trees*

Bare-root liners of *Acer ×freemanii* 'Jeffersred', *A. rubrum* 'Franksred', *Liriodendron tulipifera*, *Platanus acerifolia* 'Bloodgood', *Quercus rubra*, *Ulmus japonica × wilsoniana* 'Morton', and *U.* Triumph™ were grown in 95-L containers using 85% pine bark: 15% peat moss. One of four different fertilization levels (1.05, 2.11, 3.16, or 4.20 $g{\cdot}L^{-1}$ in 2006; levels increased by 25% in 2007) was applied to each tree, and the growth and physiological responses were examined over the course of two growing seasons. We hypothesized that growth is driven by leaf area, and leaf area is increased with fertilizer addition.

Height and caliper growth were small in 2006 compared to 2007, likely due to transplant shock and lower fertilization rates compared to the second season. Fertilization resulted in increased caliper growth with applications up to 3.95 $g \cdot L^{-1}$ container for all species. Total leaf area (TLA) also increased with increased fertilization up to 3.95 $g \cdot L^{-1}$. The relationship between caliper and TLA supports the hypothesis that an increase in TLA is associated with increased growth.

Photosynthetic rate ($A_{max}$) was unaffected by fertilizer addition in 2006, and decreased at the highest level of fertilizer in 2007. Fertilizer reduced leaf conductance to water vapor ($g_{wv}$) for both years. The negative response of $A_{max}$ and $g_{wv}$ to fertilizer in 2007, and their negative correlation with TLA, suggests that the greater leaf area resulted in greater water stress, preventing the trees from reaching their full photosynthetic potential. This conclusion is supported by midday stem water potential ($\Psi_w$), which was lower for the 5.26 $g \cdot L^{-1}$ fertilizer treatment compared to the 1.32 $g \cdot L^{-1}$ treatment. Water use efficiency (WUE; $A_{max}/g_{wv}$) increased with addition of fertilizer, perhaps mediating some of the effects of water stress.

From a practical standpoint growers should note that fertilizer applications up to approximately 3.95 $g \cdot L^{-1}$ can increase growth, but water availability must be managed carefully to avoid the water stress associated with increased leaf area.

Nursery managers are interested in indirect measurements as a quick and simple method for monitoring the nutrient status of their crops. Results from previous research has shown some potential in correlating chlorophyll fluorescence ($F_v/F_m$) or SPAD values with nitrogen concentration (Daughtry et al., 2000; Groninger et al., 1996; Krause and Weis, 1991; Loh et al., 2002; Parent et al., 2005).The results from this study indicate that while SPAD values are correlated with N concentration, the relationships are species specific and may only be useful in detecting nutrient deficiencies which are already visibly noticeable. No correlations were found between $F_v/F_m$ and N concentration.

*Conifers*

Seedlings (2+2 or plug+2) of *Abies fraseri*, *Picea glauca* var. *densata*, *P. pungens glauca*, and *Pinus strobus* were grown in 10.2-L containers using 90% pine bark: 10% peat moss, 80%:20% or 70%:30%. In addition, a fertilizer application of 2, 4, or 8 $g{\cdot}L^{-1}$ was applied to each tree. The goal of this study was to indentify substrate and nutrition levels that result in optimal growth and appearance for landscape conifers and living Christmas trees.

In 2006, stem caliper increased with the addition of 8 $g{\cdot}L^{-1}$ fertilizer compared to the two lower treatments. During the second season, addition of 4 or 8 $g{\cdot}L^{-1}$ fertilizer increased conifer height compared to 2 $g{\cdot}L^{-1}$; caliper differences between treatments were not observed in 2007. Stem caliper was greater for trees grown in the 80% pine bark: 20% peat moss combination compared to the 90:10 or 70:30 combinations; height and volume were not affected by media. Since the 80:20 media combination resulted in sufficient growth for all the species tested, nursery managers could potentially use the same media combination for multiple species rather than having to make or purchase separate mixes for each species.

Photosynthesis rates for the single-needle conifers were generally greater at higher fertilizer levels in 2006; however, during the 2007 season, $A_{max}$ for all species was either not affected or was lower at higher fertilizer rates. Media moisture levels were lower with addition of 4 or 8 $g{\cdot}L^{-1}$ fertilizer compared to 2 $g{\cdot}L^{-1}$, which implies that the growth increase in response to higher fertilizer applications resulted in greater water stress and depressed photosynthesis rates. Water use efficiency (WUE; $A_{max}/g_{wv}$) was greater with higher fertilizer additions for all species in 2007. The media containing 30% peat moss had generally higher moisture content than the combination containing 10% peat moss. Once again, growers are cautioned to carefully monitor the water management practices in conjunction with fertilizer addition and media composition to ensure that trees receive adequate levels of both resources.

Foliar N concentrations were positively associated with volume growth, but critical N levels were species dependent. Chlorophyll fluorescence levels were not associated with N concentration or $A_{max}$ in 2006, and were negatively correlated with N concentration in 2007. Growers are cautioned not to rely on such measurements without developing specific protocols for their crops and site conditions. Critical foliar N levels are generally consistent with previous studies (Rothstein and Lisuzzo, 2006; Slesak and Briggs, 2007); foliar N concentration should be 1.8% or greater for *A. fraseri* and *P. strobus* and no less than 2 – 2.5% for the *P. glauca* var. *densata* and *P. pungens glauca* when tested in late summer.

**Future Research**

Results from this study indicate that the optimal rate of fertilizer addition is approximately 4 $g \cdot L^{-1}$ for the landscape shade trees tested and between 4 and 8 $g \cdot L^{-1}$ for the conifer species, which is in agreement with conventional recommendations. However, with increased water management, greater rates of fertilizer application could further increase growth beyond the levels observed in this study. Studies on cyclic irrigation have already shown the potential of increasing plant growth by applying water periodically throughout the day compared to a single application (Fain et al., 1998; Ruter, 1998).

Additional studies examining a broader scale of fertilization levels may also be useful in detecting correlations between $F_v/F_m$ or SPAD measurements and N concentrations. Furthermore, examining the relationships over a variety of soil or media types and environmental conditions would aid in developing specific recommendations for growers across the country.

## Literature Cited

Daughtry, C.S.T., C.L. Walthall, M.S. Kim, E.B. de Colstoun, and J.E. McMurtre. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sensing Environ. 74:229-239.

Fain, G.B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1998. Effects of cyclic micro-irrigation and substrate in pot-in-pot production. J. Environ. Hort. 16:215-218.

Groninger, J.W., J.R. Seiler, J.A. Peterson, and R.E. Kreh. 1996. Growth and photosynthetic responses of four Virginia piedmont tree species to shade. Tree Phys. 16:773-778.

Krause, GH and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Mol Biol. 42: 313-349.

Loh, F.C.W., J.C. Grabosky, and N.L. Bassuk. 2002. Using the SPAD 502 meter to assess chlorophyll and nitrogen content of Benjamin fig and cottonwood leaves. HortTech. 12:682-686.

Mathers, H.M. 2003. Summary of temperature stress issues in nursery containers and current methods of protection. HortTech. 13(4):617-624.

Neal, C. 2004. Production systems for small trees and shrubs in New Hampshire. University of New Hampshire Cooperative Extension. 17 December 2007. http://extension .unh.edu/agric/AGNLT/PSSTSNH.htm

Parent, L.E., L. Khiari, and A. Pettigrew. 2005. Nitrogen diagnosis of Christmas tree needle greenness. Can. J. Plant Sci. 85:939-947.

Rothstein, D.E. and N.J. Lisuzzo. 2006. Optimal nutrition and diagnosis of *Abies fraseri* Christmas trees in Michigan. North. J. Applied For. 23(2):106-113.

Ruter, J.M. 1998. Pot-in-pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. J. Environ. Hort. 16:159-162.

Ruter, J.M. 1995. Production system and copper hydroxide influences on growth and photosynthesis of *Magnolia grandiflora* 'St. Mary'. HortScience. 30:795.

Ruter, J.M. 1993. Growth and landscape performance of three landscape plants produced in conventional and pot-in-pot production systems. J. Environ. Hort. 11(3):124-27.

Slesak, R.A. and R.D. Briggs. 2007. Christmas tree response to N fertilization and the development of critical foliar N levels in New York. North. J. Appl. For. 24(3):209-217.

## APPENDIX 1

Table A1.1. Mean separation for nitrogen concentration (%) in foliar samples collected from seven taxa of deciduous shade tree grown in a PIP production system under four fertilizer rates in 2006.

| Trt† | AF | AR | LT | PA | QR | UJ | UT |
|---|---|---|---|---|---|---|---|
| 0.15 g | 2.300a | 2.368a | 2.256a | 1.976a | 2.562a | 2.520a | 2.494a |
| 0.30 g | 2.368ab | 2.446ab | 2.782b | 2.232b | 2.705ab | 2.798b | 2.810b |
| 0.45 g | 2.562b | 2.562ab | 2.974b | 2.540c | 2.850b | 2.972bc | 3.040c |
| 0.60 g | 2.538b | 2.630b | 3.356c | 2.670c | 3.098c | 3.162c | 3.360d |

†Treatment: g $N \cdot L^{-1}$ container

Table A1.2. Mean separation for nitrogen concentration (%) in foliar samples collected from seven taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer rates in 2007.

| Trt† | AF | AR | LT | PA | QR | UJ | UT |
|---|---|---|---|---|---|---|---|
| 0.19 g | 1.570a | 1.680a | 1.862a | 1.718a | 2.084a | 1.998a | 1.630a |
| 0.38 g | 2.002b | 2.196b | 2.316b | 2.148b | 2.560b | 2.240b | 2.140b |
| 0.56 g | 2.245c | 2.206b | 2.606c | 2.344bc | 2.796bc | 2.608c | 2.592c |
| 0.75 g | 2.508d | 2.682c | 2.798c | 2.512c | 2.990c | 2.742c | 2.782c |

†Treatment: g $N \cdot L^{-1}$ container

Table A1.3. Pearson's correlation values for total height growth, total caliper growth, total volume growth, total leaf area (TLA), photosynthetic rate ($A_{max}$), molar transpiration (E), relative chlorophyll content (SPAD), 7 essential elements, pH, and nitrate-N ($NO_3$) in pour-thru leachate of seven taxa of deciduous shade tree grown in a pot-in-pot production system under four fertilizer rates in Michigan during the 2006 and 2007 growing seasons.

| | Cal. | Vol. | TLA | $A_{max}$ | E | SPAD | N | P |
|---|---|---|---|---|---|---|---|---|
| Ht. | 0.05 | 0.16** | -0.17*** | 0.15** | 0.10* | 0.06 | 0.23*** | 0.01 |
| Cal. | | 0.89*** | 0.58*** | -0.04 | -0.02 | -0.03 | 0.05 | 0.16** |
| Vol. | | | 0.68*** | 0.02 | 0.02 | -0.05 | 0.09 | 0.12* |
| TLA | | | | -0.21*** | -0.24*** | 0.03 | 0.13*** | 0.09 |
| $A_{max}$ | | | | | 0.75*** | 0.13*** | -0.28*** | -0.20*** |
| E | | | | | | 0.12*** | -0.39*** | -0.28*** |
| SPAD | | | | | | | -0.04 | -0.38*** |
| N | | | | | | | | 0.69*** |
| P | | | | | | | | |
| K | | | | | | | | |
| Mg | | | | | | | | |
| Ca | | | | | | | | |
| Fe | | | | | | | | |
| Cu | | | | | | | | |
| pH | | | | | | | | |

continued

| | K | Mg | Ca | Fe | Cu | pH | $NO_3$ |
|---|---|---|---|---|---|---|---|
| Ht. | 0.02 | 0.11 | 0.21** | 0.11 | 0.08 | -0.29*** | 0.28*** |
| Cal. | 0.01 | -0.41*** | -0.53*** | -0.31*** | -0.10 | 0.00 | 0.13 |
| Vol. | 0.00 | -0.40*** | -0.57*** | -0.42*** | -0.06 | 0.02 | 0.18** |
| TLA | 0.10* | -0.41*** | -0.56*** | -0.44*** | -0.15** | -0.02 | 0.20*** |
| $A_{max}$ | -0.25*** | 0.06 | 0.22*** | 0.18** | 0.21*** | 0.16** | -0.25*** |
| E | -0.37*** | -0.06 | 0.18** | 0.18** | 0.29*** | 0.16** | -0.16*** |
| SPAD | -0.43*** | -0.05 | 0.23*** | 0.27*** | 0.33*** | 0.10* | 0.17*** |
| N | 0.78*** | 0.43*** | -0.06 | -0.29*** | -0.42*** | -0.19*** | 0.39*** |
| P | 0.77*** | 0.29*** | -0.20*** | -0.23*** | -0.41*** | -0.20* | 0.27*** |
| K | | 0.37*** | -0.28*** | -0.37*** | -0.56*** | 0.05 | 0.13 |
| Mg | | | 0.61*** | 0.21*** | -0.15** | -0.05 | -0.03 |
| Ca | | | | 0.59*** | 0.31*** | -0.12 | -0.04 |
| Fe | | | | | 0.36*** | -0.05 | -0.24** |
| Cu | | | | | | -0.11 | -0.11 |
| pH | | | | | | | -0.32*** |

## APPENDIX 2

Table A2.1. Means (±SE) for total height growth (cm) during the 2006 and 2007 growing seasons for four species of conifer grown in a PIP production system under a 3 × 3 factorial treatment of fertilizer level (2, 4, or 8 g fertilizer $L^{-1}$) and media type (ratios of pine bark:peat moss; v:v).

| | *Abies fraseri* | | *Picea glauca* var. *densata* | | *Picea pungens glauca* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|
| treatment | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| 20 g, 90:10 | 7.00 (±1.06) | 2.57 (±0.52) | 6.50 (±0.69) | 5.60 (±0.93) | 5.61 (±0.70) | 5.80 (±0.96) | 22.83 (±2.51) | 8.55 (±1.60) |
| *2 g, 80:20* | *8.06 (±1.31)* | *6.67 (±1.38)* | *7.00 (±0.83)* | *6.17 (±0.69)* | *6.11 (±1.03)* | *5.35 (±0.73)* | *14.06 (±1.77)* | *14.17 (±2.36)* |
| **2 g, 70:30** | **8.06 (±1.80)** | **6.60 (±1.02)** | **6.00 (±1.21)** | **7.10 (±0.74)** | **6.57 (±1.71)** | **9.40 (±1.00)** | **16.30 (±2.35)** | **9.80 (±1.92)** |
| 4 g, 90:10 | 8.56 (±1.32) | 12.31 (±1.47) | 5.90 (±1.24) | 8.15 (±1.07) | 7.22 (±0.90) | 12.70 (±1.44) | 22.86 (±3.04) | 19.65 (±2.08) |
| *4 g, 80:20* | *10.56 (±1.27)* | *11.75 (±1.24)* | *7.61 (±0.48)* | *12.75 (±1.94)* | *10.56 (±1.78)* | *11.85 (±1.68)* | *12.56 (±2.03)* | *18.28 (±1.60)* |
| **4 g, 70:30** | **10.86 (±1.87)** | **15.43 (±2.00)** | **6.50 (±0.88)** | **12.33 (±0.96)** | **7.75 (±0.90)** | **15.25 (±1.57)** | **21.64 (±2.84)** | **16.40 (±1.85)** |
| 8 g, 90:10 | 8.44 (±1.50) | 13.28 (±2.24) | 8.33 (±1.27) | 11.85 (±1.32) | 10.56 (±2.77) | 15.00 (±1.38) | 14.56 (±2.39) | 18.67 (±2.68) |
| *8 g, 80:20* | *11.50 (±1.87)* | *16.78 (±2.60)* | *8.45 (±1.23)* | *14.15 (±1.45)* | *8.00 (±1.68)* | *12.65 (±1.80)* | *15.72 (±1.86)* | *21.69 (±1.89)* |
| **8 g, 70:30** | **7.67 (±1.01)** | **13.50 (±4.59)** | **7.78 (±0.93)** | **13.30 (±1.48)** | **8.78 (±1.79)** | **15.98 (±1.45)** | **20.22 (±1.65)** | **21.55 (±2.16)** |

Table A2.2. Means (±SE) for total caliper growth (mm) during the 2006 and 2007 growing seasons for four species of conifer grown in a PIP production system under a 3 × 3 factorial treatment of fertilizer level (2, 4, or 8 g fertilizer $L^{-1}$) and media type (ratios of pine bark:peat moss; v:v).

| | *Abies fraseri* | | *Picea glauca* var. *densata* | | *Picea pungens glauca* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|
| treatment | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| 2 g, 90:10 | 2.95 (±0.30) | 4.83 (±1.02) | 1.62 (±0.60) | 7.06 (±0.65) | 2.53 (±0.41) | 7.10 (±0.52) | 2.57 (±0.36) | 4.95 (±0.49) |
| *2 g, 80:20* | *2.31 (±0.34)* | *5.00 (±0.46)* | *1.61 (±0.45)* | *7.32 (±0.61)* | *1.83 (±0.19)* | *8.56 (±1.06)* | *2.12 (±0.42)* | *8.09 (±0.86)* |
| **2 g, 70:30** | **2.11 (±0.37)** | **5.64 (±0.72)** | **1.87 (±0.16)** | **7.68 (±0.57)** | **3.66 (±0.73)** | **9.48 (±1.61)** | **2.33 (±0.29)** | **4.57 (±0.66)** |
| 4 g, 90:10 | 2.26 (±0.14) | 7.23 (±0.51) | 1.83 (±0.20) | 9.06 (±0.60) | 3.37 (±0.30) | 9.55 (±0.67) | 2.29 (±0.38) | 11.49 (±0.94) |
| *4 g, 80:20* | *3.57 (±0.31)* | *7.85 (±0.54)* | *2.54 (±0.24)* | *9.52 (±0.45)* | *3.26 (±0.57)* | *10.70 (±1.49)* | *3.43 (±0.44)* | *10.29 (±0.65)* |
| **4 g, 70:30** | **3.81 (±0.61)** | **8.50 (±0.68)** | **2.69 (±0.35)** | **9.46 (±0.48)** | **4.45 (±0.45)** | **9.65 (±0.88)** | **2.32 (±0.40)** | **10.01 (±1.05)** |
| 8 g, 90:10 | 4.54 (±0.34) | 7.97 (±0.61) | 4.55 (±0.40) | 8.42 (±0.58) | 4.17 (±0.37) | 9.37 (±0.57) | 2.83 (±0.85) | 9.46 (±0.66) |
| *8 g, 80:20* | *4.41 (±0.54)* | *8.74 (±0.74)* | *4.81 (±0.43)* | *9.42 (±0.57)* | *5.24 (±0.54)* | *11.55 (±1.02)* | *3.54 (±0.41)* | *10.11 (±1.40)* |
| **8 g, 70:30** | **5.06 (±0.28)** | **10.40 (±1.09)** | **4.59 (±0.38)** | **9.41 (±0.54)** | **6.28 (±0.68)** | **10.81 (±1.01)** | **4.11 (±0.67)** | **10.20 (±0.47)** |

Table A2.3. Means (±SE) for total volume growth index ($cm^3$) during the 2006 and 2007 growing seasons for four species of conifer grown in a PIP production system under a 3 × 3 factorial treatment of fertilizer level (2, 4, or 8 g fertilizer $L^{-1}$) and media type (ratios of pine bark:peat moss, v:v).

| | *Abies fraseri* | | *Picea glauca* var. *densata* | | *Picea pungens glauca* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|
| treatment | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 | 2006 | 2007 |
| 2 g, 90:10 | 0.68 (±0.19) | 0.77 (±0.27) | 0.39 (±0.30) | 3.03 (±0.69) | 0.48 (±0.16) | 3.15 (±0.72) | 1.65 (±0.53) | 2.77 (±0.85) |
| *2 g, 80:20* | *0.53 (±0.16)* | *2.07 (±0.81)* | *0.29 (±0.17)* | *3.61 (±0.95)* | *0.21 (±0.05)* | *4.95 (±1.27)* | *0.90 (±0.31)* | *11.51 (±3.62)* |
| **2 g, 70:30** | **0.56 (±0.23)** | **2.55 (±0.86)** | **0.18 (±0.04)** | **4.52 (±0.72)** | **1.27 (±0.56)** | **10.59 (±4.42)** | **1.25 (±0.39)** | **3.07 (±1.27)** |
| 4 g, 90:10 | 0.48 (±0.11) | 6.22 (±0.85) | 0.29 (±0.13) | 7.18 (±1.23) | 0.95 (±0.20) | 12.68 (±2.55) | 1.37 (±0.38) | 26.67 (±4.40) |
| *4 g, 80:20* | *1.45 (±0.31)* | *8.34 (±2.41)* | *0.56 (±0.15)* | *12.22 (±2.80)* | *1.50 (±0.51)* | *18.87 (±7.15)* | *1.59 (±0.37)* | *19.77 (±2.54)* |
| **4 g, 70:30** | **2.28 (±1.00)** | **11.62 (±2.09)** | **0.57 (±0.15)** | **11.50 (±2.01)** | **1.76 (±0.40)** | **15.58 (±3.21)** | **1.25 (±0.35)** | **18.00 (±3.91)** |
| 8 g, 90:10 | 1.84 (±0.43) | 9.02 (±2.21) | 1.80 (±0.39) | 9.25 (±1.65) | 2.09 (±0.64) | 13.49 (±1.95) | 1.91 (±1.05) | 19.07 (±4.35) |
| *8 g, 80:20* | *2.85 (±0.90)* | *14.20 (±2.96)* | *2.43 (±0.69)* | *13.75 (±2.67)* | *2.99 (±1.02)* | *17.65 (±3.41)* | *2.06 (±0.46)* | *23.57 (±4.80)* |
| **8 g, 70:30** | **2.09 (±0.46)** | **15.67 (±6.19)** | **1.79 (±0.35)** | **12.55 (±2.45)** | **3.90 (±1.29)** | **21.74 (±7.00)** | **4.24 (±1.03)** | **21.76 (±2.03)** |

Table A2.4: Summary analysis of variance for foliar mineral content in needles from *Abies fraseri*, *Picea glauca* var. *densata*, *Picea pungens glauca*, and *Pinus strobus* grown in a PIP production system under three fertilizer levels and three media combinations.

| Source of variation | | F-values | | | | |
|---|---|---|---|---|---|---|
| | d.f. | N | P | K | Mg | Mn |
| between subjects | | | | | | |
| Species (Spp) | 3 | 155.46*** | 36.90*** | 12.40*** | 3.93* | 40.08*** |
| Fertilizer (F) | 2 | 399.29*** | 8.73** | 0.63 | 4.29* | 49.42*** |
| Spp × F | 6 | 11.83*** | 1.23 | 3.13** | 1.37 | 6.81*** |
| Block (Blk) | 9 | 3.21** | 1.53 | 1.36 | 2.41* | 3.41** |
| Blk × Spp | 27 | 1.38 | 1.49 | 2.04** | 2.30** | 0.95 |
| within subjects | | | | | | |
| Year (Yr) | 1 | 252.14*** | 14.81** | 6.43* | 28.19*** | 0.09 |
| Spp × Yr | 3 | 9.09*** | 21.42*** | 13.29*** | 3.47* | 0.54 |
| F × Yr | 2 | 32.47*** | 1.91 | 1.09 | 2.66 | 48.47*** |
| Spp × F × Yr | 6 | 0.99 | 0.99 | 1.20 | 0.90 | 0.70 |
| Blk × Spp × F | 72 | 0.65 | 0.78 | 0.84 | 0.76 | 1.09 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

Table A2.5: Summary analysis of variance for chlorophyll fluorescence ($F_v/F_m$) of needles for *Picea glauca* var. *densata*, *Picea pungens glauca*, *Abies fraseri* and *Pinus strobus* grown in a PIP production system under three fertilizer levels and three media combinations.

| Source of variation | d.f. | F-values $F_v/F_m$ |
|---|---|---|
| Between subjects | | |
| Species (Spp) | 3 | 13.20*** |
| Fertilizer (F) | 2 | 17.17*** |
| Spp × F | 6 | 2.23* |
| Media (M) | 2 | 0.12 |
| SPP × M | 6 | 2.74* |
| F × M | 4 | 1.87 |
| Spp × F × M | 12 | 1.43 |
| Block (Blk) | 9 | 4.31** |
| Blk × Spp | 27 | 2.79** |
| Blk × Spp × F | 72 | 0.78 |
| Within subjects | | |
| Date (D) | 6 | 73.65*** |
| Spp × D | 13 | 25.86*** |
| F × D | 12 | 0.86 |
| Spp × F × D | 26 | 1.28 |
| M × D | 12 | 1.24 |
| Spp × M × D | 26 | 0.78 |
| F × M × D | 24 | 0.81 |
| Spp × F × M × D | 52 | 0.72 |
| Blk × Spp × F × M | 205 | 0.79 |

Table A2.6: Summary analysis of variance for nitrate-N ($NO_3$) concentration, pH, and electric conductivity (EC) in pour-thru leachate samples for *Abies fraseri* and *Pinus strobus* with fertilizer × media combinations of: 2 $g \cdot L^{-1}$ × 90B:10PM, 2 $g \cdot L^{-1}$ × 70B:30PM, 4 $g \cdot L^{-1}$ × 80B:20PM, 8 $g \cdot L^{-1}$ × 90B:10PM, and 8 $g \cdot L^{-1}$ × 70B:30PM.

| Source of variation | d.f.† | F-values | d.f.† | F-values | |
|---|---|---|---|---|---|
| | | $NO_3$ | | pH | EC |
| Between subjects | | | | | |
| Species (Spp) | 1 | 12.21* | 1 | 5.37 | 3.96 |
| Fertilizer (F) | 1 | 560.83*** | 1 | 73.55*** | 269.84*** |
| Spp × F | 1 | 7.25* | 1 | 0.09 | 0.84 |
| Media (M) | 1 | 1.08 | 1 | 4.08 | 2.13 |
| SPP × M | 1 | 1.23 | 1 | 6.58* | 0.28 |
| F × M | 1 | 0.17 | 1 | 1.63 | 0.07 |
| Spp × F × M | 1 | 2.54 | 1 | 0.02 | 3.37 |
| Block (Blk) | 4 | 2.20 | 4 | 1.92 | 0.86 |
| Blk × Spp | 4 | 1.17 | 4 | 0.64 | 1.41 |
| Blk × Spp × F | 16 | 0.87 | 16 | 1.31 | 2.09 |
| Within subjects | | | | | |
| Date (D) | 10 | 82.34*** | 9 | 149.83*** | 167.10*** |
| Spp × D | 9 | 2.14* | 8 | 1.03 | 4.23*** |
| F × D | 10 | 1.74 | 9 | 4.22*** | 14.95*** |
| Spp × F × D | 9 | 3.20** | 8 | 0.38 | 0.98 |
| M × D | 10 | 2.68** | 9 | 8.36*** | 0.25 |
| Spp × M × D | 9 | 1.44 | 8 | 2.07* | 0.85 |
| F × M × D | 10 | 0.64 | 9 | 0.50 | 2.41* |
| Spp × F × M × D | 9 | 0.42 | 8 | 1.14 | 0.42 |
| Blk × Spp × F × M | 16 | 1.54 | 14 | 1.14 | 1.17 |

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p < 0.0001$

†degrees of freedom do not represent all possible fertilizer × media combinations sampled due to statistical limitations.

Table A2.7. Pearson's correlation values for total height growth, caliper relative growth rate (RGR), total volume growth (vol), photosynthetic rate ($A_{max}$), conductance ($g_{wv}$), molar transpiration (E), chlorophyll fluorescence ($F_v/F_m$), 7 essential elements, pH, and nitrate-N ($NO_3$) in pour-thru leachate for conifers grown in a PIP production system under three fertilizer levels and three media combinations.

| | RGR | vol | $A_{max}$ | $g_{wv}$ | E | $F_v/F_m$ | N | P |
|---|---|---|---|---|---|---|---|---|
| height | 0.15*** | 0.58*** | -0.29*** | -0.34*** | -0.43*** | 0.01 | -0.02 | -0.24*** |
| RGR | | 0.35*** | 0.05* | 0.02 | -0.09*** | 0.18*** | 0.41*** | 0.11*** |
| vol | | | -0.06** | -0.14*** | -0.23*** | 0.01 | 0.21*** | -0.15*** |
| $A_{max}$ | | | | 0.69*** | 0.72*** | 0.09*** | 0.32*** | 0.22*** |
| $g_{wv}$ | | | | | 0.84*** | 0.06** | 0.36*** | 0.34*** |
| E | | | | | | 0.10*** | 0.32*** | 0.32*** |
| $F_v/F_m$ | | | | | | | 0.06** | -0.01 |
| N | | | | | | | | 0.30*** |
| P | | | | | | | | |
| K | | | | | | | | |
| Mg | | | | | | | | |
| B | | | | | | | | |
| Mn | | | | | | | | |
| Cu | | | | | | | | |
| pH | | | | | | | | |

(continued)

| | K | Mg | B | Mn | Cu | pH | $NO_3$ |
|---|---|---|---|---|---|---|---|
| height | 0.02 | 0.04 | 0.28*** | 0.18*** | -0.28*** | -0.10* | 0.05 |
| RGR | -0.06* | -0.02 | 0.28*** | 0.24*** | -0.38*** | -0.39*** | 0.25*** |
| volume | -0.03 | -0.01 | 0.21*** | 0.16*** | -0.36*** | -0.34*** | 0.32*** |
| $A_{max}$ | -0.14*** | -0.07** | -0.32*** | 0.16*** | 0.07** | -0.12** | 0.06 |
| $g_{wv}$ | -0.17*** | -0.18*** | -0.38*** | 0.21*** | 0.06* | -0.02 | 0.02 |
| E | -0.27*** | -0.23*** | -0.51*** | 0.25*** | 0.18*** | 0.20*** | -0.08* |
| $F_v/F_m$ | -0.10*** | -0.05* | -0.05 | 0.07** | 0.00 | 0.03 | -0.07 |
| N | -0.24*** | -0.28*** | 0.21*** | 0.59*** | -0.27*** | -0.45*** | 0.51*** |
| P | 0.54*** | 0.02 | 0.13*** | 0.24*** | -0.11*** | -0.29*** | 0.33*** |
| K | | 0.18*** | 0.28*** | -0.23*** | -0.15*** | -0.25*** | 0.28*** |
| Mg | | | 0.34*** | -0.24*** | -0.01 | -0.10* | -0.18*** |
| B | | | | 0.13*** | -0.40*** | -0.52*** | 0.36*** |
| Mn | | | | | -0.30*** | -0.23*** | 0.54*** |
| Cu | | | | | | 0.40*** | -0.28*** |
| pH | | | | | | | -0.43*** |