This is to certify that the
thesis entitled

GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES AND CONIFERS IN RESPONSE TO CYCLIC IRRIGATION REGIMES

presented by

AMANDA JO TAYLOR

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Horticulture

Major Professor's Signature

6/21/2010

Date

GROWTH AND PHYSIOLOGY
OF DECIDUOUS SHADE TREES AND CONIFERS
IN RESPONSE TO CYCLIC IRRIGATION REGIMES

By

Amanda Jo Taylor

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Horticulture

2010

ABSTRACT

GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES AND CONIFERS IN RESPONSE TO CYCLIC IRRIGATION REGIMES

By

Amanda Jo Taylor

Nursery water management is becoming increasingly important as input costs rise and water use regulations increase. Irrigation regimes consisting of varying combinations of irrigation rates and cycle frequencies were tested to investigate their effect on the growth and physiology of PIP-grown conifers and deciduous shade trees. The project consisted of two studies: 1) examining the effects of cyclic irrigation and reduced irrigation rates on seven taxa of deciduous shade trees, and 2) examining the effects of cyclic irrigation and reduced irrigation rates on four conifer species. Growth and physiological responses were measured throughout two growing seasons and were more responsive to irrigation rate than cycle frequency. In the first study, we found that 50% reductions in daily irrigation rates produced conifers of similar size to those produced with the typical irrigation rate. However, the same reduction in the irrigation rate of PIP-grown deciduous shade trees decreased stem radial growth of most species tested. Applying water in multiple cycles did not affect tree growth in either study. Of the various methods of calculating WUE, carbon isotope discrimination was the most sensitive to irrigation regimes.

ACKNOWLEDGMENTS

Thank you to my advisor Bert Cregg for being so understanding and making work fun. Thanks to the Cregg family for always making me feel so welcome in their home. Thank you to my family, who supported me even though they don't understand what I'm doing or why I'm doing it. Thanks especially to Christina for talking me down from the ledge on more than one occasion. You are the wind beneath the wings.

Thank you, Heutsche family for making sure that I was fed during the many months that I was writing my thesis and for being so hospitable to Buddy. Thank you, Ted for being unselfish while I was writing and revising and for putting up with me during the process. This journey would have been less enjoyable if Aaron Warsaw and Nick Pershey were not around. Aaron – thank you for listening. Nick – thanks for making me laugh (whether you meant to or not).

Thank you to my committee members, Jeff Andresen and Tom Fernandez, for the advice.

TABLE OF CONTENTS

CHAPTER ONE
LITERATURE REVIEW

CHAPTER TWO
GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES GROWN UNDER CYCLIC IRRIGATION

CHAPTER THREE
GROWTH AND PHYSIOLOGY OF CONTAINER-GROWN CONIFERS UNDER CYCLIC IRRIGATION

CHAPTER FOUR
COMBINED SUMMARY AND CONCLUSIONS

LIST OF TABLES

LIST OF FIGURES

Introduction

Water management is a growing concern for agricultural producers because of a predicted decrease in water availability, a predicted substantial increase in water cost, stricter water use regulations, and a growing concern for the environment. The percentage of U.S. farms using irrigation increased to 86% in 2008, from 71% in 2003 (NASS, 2008); however, more than 1500 farms stopped irrigating between 2003 and 2008 due to insufficient ground or surface water supplies (NASS, 2008). In Michigan, 30% of woody plant producers ranked input costs as their primary or secondary concern for future production problems (MDA, 2010).

Agriculture is a major contributor of NO3-N to waterways, and groundwater, which provides drinking water for more than half of the U.S. population, is at risk (Cooper, 1993; Nolan et al., 1997). In temperate ecosystems, nitrogen not used by plants is converted to nitrate in soils, where it is readily leached into the underlying groundwater. Aquifers are most vulnerable in regions with excessive nitrogen contributions, porous soils, and a low ratio of woodland to cropland acres (Nolan et al., 1997). As a result, groundwater in the upper Midwest is expected to experience moderate to high nitrate contamination (Nolan and Hitt, 2006). Several states have imposed regulations limiting water consumption and discharge from agricultural operations, including nurseries, with the remaining states expected to follow suit (Beeson et al., 2004).

Production of landscape nursery crops in the United States is an intensive land use that is largely concentrated in several regions of the country, including hydrologically sensitive areas in western Michigan and northern Ohio. In these regions, production of high-value nursery crops, such as landscape shade trees and container-grown Christmas trees, is shifting from traditional field-growing systems to container production (NASS, 2007; Neal, 2004). In general, irrigation and fertilization of trees in container production systems is more intensive than in field production, creating the potential for increased environmental impacts. Because fertilizer applications represent a relatively small portion of production costs, growers may overapply N to ensure maximum growth of high-value container-grown material (Tyler et al., 1996). Limited container volumes and the low nutrient and water holding capacities of commonly used, pine bark-based substrates also contribute to the need for frequent irrigation and N applications and may cause substantial leaching from containers.

Historically, irrigation practices have been based on anecdotal evidence. Growers often irrigate excessively in an effort to ensure maximal growth. Irrigation is often applied only once a day, in the morning hours when there is little risk of sun scorch or pathogen dispersal and infection. The use of cyclic irrigation, or applying subvolumes of a plant's daily water allowance in multiple allotments throughout the day, can increase irrigation efficiency (Ismail et al., 2007; Warren and Bilderback, 2005). Improved irrigation efficiency is one way in which water can be conserved and water quality can be maintained; however, only water-conserving irrigation regimes which are not detrimental to crop growth and quality will be implemented by growers.

In the southeastern U.S., previous studies have documented that cyclic irrigation can be beneficial to tree growth and physiology; however, there has been little research testing irrigation methods in the temperate climate of the northern U.S. The objectives of this study were to document the effects of water-conserving irrigation regimes on soil moisture fluctuations and the resulting growth and underlying physiological mechanisms of container-grown coniferous and broadleaf trees. These results will provide a valid scientific basis on which irrigation guidelines for growers in the upper Midwest can be developed.

LITERATURE CITED

Beeson, Jr., R. C., M. A. Arnold, T.E. Bilderback, B. Bolusky, S. Chandler, H.M. Gramling, J.D. Lea-Cox, J. R. Harris P.J. Klinger, H.M. Mathers, J.M. Ruter, and T.H. Yeager. 2004. Strategic vision of container nursery irrigation in the next ten years. Journal of Environmental Horticulture 22(2): 113-115.

Cooper, C.M. 1993. Biological effects of agriculturally derived surface water pollutants on aquatic systems – a review. Journal of Environmental Quality 22:402-408.

Ismail, S.M., K. Ozawa, and N.A. Khondaker. 2007. Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. First Power). Agricultural Water Management 95(2008): 116-122.

MDA. 2010. Generally accepted agricultural and management practices for irrigation water use. Michigan Department of Agriculture. 15 February 2010. <http://michigan.gov/documents/MDA_Irrigation_GAAMP_129710_7.pdf>.

NASS. 2007. Nursery crops summary 2006. National Agricultural Statistics Service and United States Department of Agriculture. 12 December 2010. <http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plans>.

NASS. 2008. 2007 Census of agriculture farm and ranch irrigation survey report. National Agricultural Statistics Service and United States Department of Agriculture. 20 January 2010. <http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Farm_and_Ranch_Irrigation_Survey/fris08.pdf>.

Neal, C. 2004. Production systems for small trees and shrubs in New Hampshire. University of New Hampshire Cooperative Extension. 18 December 2010. <http://extension.unh.edu/agric/AGNLT/PSSTSNH.htm>.

Nolan, B.T., B.C. Ruddy, K.J. Hitt, and D.R. Helsel. 1997. Risk of nitrate in groundwaters of the United States – a national perspective. Environ. Sci. Technol. 31:2229-2236.

Nolan, B.T. and K.J. Hitt. 2006. Vulnerability of shallow groundwater and drinking water wells to nitrate in the United States. Environ. Sci. Technol. 40:7834-7840

Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Cyclic irrigation increases irrigation application efficiency and decreases ammonium losses. Journal of Environmental Horticulture 14(4): 194-198.

Warren, S.L. and T.E. Bilderback. 2005. More plant per gallon: Getting more out of your water. Horttechnology 15(1): 14-18.

CHAPTER ONE

LITERATURE REVIEW

Literature Review

Introduction

Increased water costs, decreased water availability due to increased competition for water resources with urban areas, and anticipation of stricter water withdrawal and discharge regulations have caused nursery producers to rethink water use practices (Beeson et al., 2004). Growers commonly overwater, applying a fixed rate of irrigation exceeding plant water use; this often results in excessive water withdrawals, leaching of nutrients and chemicals into surface and ground water. The development of irrigation programs which conserve water without sacrificing tree growth and quality will allow the nursery industry to adapt to future water use regulations, increased input costs, and decreased water availability.

This report reviews research addressing the following topics related to nurseries and their irrigation practices and plant growth and physiological responses to water availability.

1. Container nurseries and irrigation
    - Pot-in-Pot production systems
    - Irrigation scheduling
    - Overhead, micro-irrigation, and cyclic irrigation
    - Movement of water through soils and substrates
    - Water application efficiency
    - Leaching fraction
2. Growth response to water conserving irrigation regimes
    - Growth response to cyclic irrigation

Growth response to reduced irrigation rates

3. Physiology of water stress

    Basic water stress physiology

    Water use efficiency

    Chlorophyll Fluorescence

    Specific leaf area

    Plant nutrition and chlorophyll content

    Water potential

Container Nurseries and Irrigation

Nursery production is an economically important sector of the U.S. agriculture industry and has grown in recent years. Nationwide, gross receipts of nursery and greenhouse crops totaled $4.65B in 2006, up 17% from 2003. Deciduous shade trees and coniferous evergreens accounted for 13% and 12% of all sales, respectively. The wholesale value of Christmas tree sales totaled $210,532 million in 2006, up more than 15% from 2003. Additionally, the quantity of Christmas trees sold by the 17 producing states increased 12% in 2006, to 11.6 million, from 10.3 million in 2003 (NASS, 2007). Within the nursery industry, container production, which demands more water than field production, is becoming more common relative to balled-and-burlapped sales, driven by a shift in customer preference (NASS, 2007).

Michigan is one of the top nursery stock producing states, with over 45,000 acres in production and supporting over 261,000 employees (Hall et al., 2005; NASS, 2007). Michigan's nursery industry is the third-ranked sector in state agricultural market value and represents a substantial source of revenue for the state, contributing 10.8% of total sales (NASS, 2007). Michigan also ranks third in the United States in cut Christmas tree production, with over 28,000 acres in production (NASS, 2007). In 2006, 17 million gallons of water per day (MGD) were withdrawn to irrigate the state's nursery and greenhouse crops (MDEQ, 2006).

Even in states with a seemingly endless supply of water, like Michigan, water availability is a growing concern for plant producers, especially those growing material in high-cost container production (Haydu and Beeson, 1997; NASS, 2007). In Michigan, 30% of woody plant producers ranked input costs as their primary or secondary concern for future production problems (MDA, 2010). Several states have imposed regulations limiting water withdrawal by nurseries (Beeson et al., 2004); in anticipation of stricter water use regulations, decreasing water availability, and increased cost, nurseries are seeking water conserving irrigation methods.

Irrigation is crucial to optimizing plant growth and quality in container production due to the limited storage capacity of containers and the highly porous substrates, especially at the height of the growing season when plant water demand is at its peak. The frequent use of pine bark-based substrates in the eastern U.S. presents growers with water and nutrient management challenges. The relatively large particle sizes make pine bark-based substrates highly porous, and therefore low in water and nutrient retention capacity. To compensate, growers apply high volumes of water and high rates of fertilizer in an attempt to maximize production. The resulting, and often highly concentrated, container solution is highly susceptible to leaching (Bilderback, 2001). Increasingly, nurseries are capturing the effluent and reusing it to irrigate (Fain et al., 2000); however, the quality of recycled water may not be appropriate for crops. Water quality is cited as the most critical factor in producing container grown plants (Yeager et al., 2007). Recycling irrigation water and stricter regulation of water quality make water management a prime concern for growers.

Nurseries can increase irrigation efficiency to decrease water use and prevent leaching of chemicals. On average, daily irrigation rates are between 1.25 and 2.5 cm of irrigation for container grown woody ornamental plants (Fain et al., 2000; Fare et al., 1992); however, because irrigation efficiency is not regularly monitored (Fain et al., 2000; Schoene et al., 2006), actual volumes applied can vary greatly. In a study by Fain et al. (2000), the application thought by growers to be 2.5 cm was actually 1.5 cm, 40% less than what was assumed.

Several agricultural agencies and horticultural organizations recognize that water management is an issue and have developed recommendations for their stakeholders. In an effort to preserve water quality and prevent pollution, Best Management Practices (BMPs) were developed in 1997 by the Southern Nursery Association (SNA) (Yeager et al., 2007) to provide guidelines for irrigation practices, capture and reuse of water, and water use monitoring. Similarly, the Michigan Department of Agriculture (MDA) has established Generally Accepted Agricultural and Management Practices (GAAMPs) for irrigation water use to promote stewardship in water management by optimizing water quantity and quality, soil and plant quality, and yield (MDA, 2010). As the nursery industry recognizes the need for and shifts toward water-conserving production practices, alternative, highly efficient production and irrigation techniques are needed.

The Pot-in-Pot Production System

The the pot-in-pot (PIP) production system is a method which increases the efficiency of crop production. Introduced in the 1990's, PIP production eliminates common problems associated with conventional above ground (CAG) container growing. Plants in CAG production often suffer from windthrow and require frequent, labor intensive repositioning, increasing production costs for growers. PIP production eliminates this problem by anchoring containers in the ground (Adrian et al., 1998). The PIP system uses two containers; a socket pot, which is sunk into the ground and serves as an anchor, and the growing pot, which is nested within the socket pot. In the summer when solar radiation is most intense, substrate temperatures in above ground containers can reach levels detrimental to plant growth. Containers in the PIP system are insulated by the surrounding soil, reducing substrate temperature fluctuations and promoting root growth (Hight and Bilderback, 1993; Ruter, 1997; Zhu et al., 2005). Pot-in-pot production also offers advantages over field production. In PIP production, less damage to roots occurs at harvest compared to field-grown trees, because the root system of a PIP-grown tree is contained within the pot (Hight and Bilderback, 1993). Field-grown trees can only be harvested when the ground is not frozen; however, trees grown in the PIP system can be harvested throughout the year, increasing the sales window in northern climates.

Irrigation Scheduling

Optimizing the timing and duration of irrigation applications is one way in which nurseries can improve irrigation efficiency. Irrigation scheduling, designed to minimize water use and optimize plant growth and quality, is defined as how much water is applied and when those applications occur (MDA, 2010). Methods used to schedule irrigation are based on plant response or substrate moisture content, which influences plant response (Jones, 2004). Some growers determine irrigation frequency by visually assessing the status of a crop or an indicator plant, a species of plant that is particularly responsive to water stress (Schoene et al., 2006); however, the subjectivity of this method can lead to inadequate irrigation. There remains a need for a simple, flexible, easily adoptable procedure for irrigation scheduling of container grown woody ornamental species that does not sacrifice plant growth or quality.

Irrigation can be scheduled using evapotranspiration (ET), which is a measure of plant water demand. ET is the sum of water evaporated and water transpired from a system. Actual evapotranspiration (ET_A) is the evapotranspiration from a given area; in the case of container production, the container and plant. This is often compared to potential or reference evapotranspiration (ET_0), the evapotranspiration from a reference crop grown under identical conditions (Beeson, 2005). Typically, that reference crop is 3” high turfgrass. In the 1940's, Penman and Monteith introduced an equation to estimate ET_0 from climatic data, taking into account irradiation, relative humidity, wind speed, and temperature (Penman, 1948; Monteith, 1964).

Modeling the water use of agronomic crops began in the 1940’s when Thornthwaite (1944) developed a model to predict crop coefficients (K_C). ET_A is species dependent, calculated as:

$$ET_A = ET_0 * K_C$$

Values of K_C, representing the ratio of ET_A:ET_0, are unique to a specific crop at a particular growth stage (Beeson, 2005). Since the introduction of the Thornthwaite model, numerous methods and models have been developed to estimate ET in nursery production from various factors including soil, irrigation method, and plant height (Fitzpatrick, 1980; Bacci et al., 2008).

Actual evapotanspiration is highly correlated with ET_0 in containerized woody ornamentals, and crop coefficients have been calculated for a number of woody ornamental species and cultivars (Beeson, 1993; Roberts and Schnipke, 1987; Knox, 1989; Bacci et al., 2008). There was no difference between ET_0 (calculated using the Thornthwaite method) and realized plant water demand of five *Acer* species grown in containers, indicating that ET_0 accurately predicts irrigation requirements of plants (Roberts and Schnipke, 1987). The use of ET to schedule irrigation of container grown woody ornamentals has been successful in producing plants of saleable size and quality (Hill and Allen, 1996), and GAAMP guidelines recommend that growers use ET data and crop coefficients, when available, to schedule irrigation (MDA, 2010).

There are minimum cumulative ET_A values required for plants to reach a marketable size and quality, indicating that restrictive water applications have the potential to reduce plant productivity and therefore, profit (Beeson, 2006). Species and plant size are major determinants of minimum water requirements for containerized nursery stock (Eakes et al., 1985; Knox, 1989). Species and cultivars of woody ornamental plants can be grouped into categories of low, moderate, or high water use (Knox, 1989; Schuch and Burger, 1997; Warsaw et al., 2009a). Container-grown plants can then be blocked by water use group and irrigated based on plant needs to avoid under- or overwatering (MDA, 2010).

Overhead Irrigation vs. Microirrigation

Water conservation in nurseries can also be achieved by transitioning to a more efficient irrigation method. In general, container-grown material is irrigated in one of two ways: overhead through sprinklers or through microsprinklers. Overhead irrigation, the traditional method used for container production, lacks application efficiency. The goal of daily irrigation in container production is to apply water to the substrate so that it is available for plant uptake, therefore, applied water that does not enter the container media is wasted. Overhead irrigation uses sprinklers mounted on risers of varying heights, based on the size of the plant material. The cylindrical shape of plant containers produces gaps, in which overhead irrigation water falls, resulting in wasted water. This inevitable off-target application and resulting low interception efficiency and water application efficiency (WAE) contributes to the low efficiency of the system. An increase in container size (larger diameter) results in larger gaps, and therefore a larger portion of applied water is lost.

Microirrigation can be used to reduce off-target applications. Microirrigation, which is commonly used for containers #7 (24.5L) and larger, is a low pressure system that increases application efficiency by reducing off-target application (Weatherspoon and Harrell, 1980; Beeson et al., 2004). Microirrigation reduces off-target application of water, because the emitter is placed close to the media surface and water is applied directly to the substrate via drip emitters or spray stakes.

The use of microirrigation is increasing in the U.S., because it uses substantially less water to increase substrate moisture content relative to overhead irrigation (NASS, 2007; Weatherspoon and Harrell, 1980). For a given area, installation costs of microirrigation are dependent on container size, whereas those of overhead irrigation installation are not. Microirrigation is more cost-efficient when growing stock in large containers because, as container size increases, and quantity of containers per area decreases, installation costs per area decrease (Haydu and Beeson, 1997).

Cyclic Irrigation

The timing and duration of irrigation events can also be manipulated to improve production system efficiency and water conservation. Cyclic, or pulse, irrigation is a method by which subvolumes of water are applied in series, in contrast to standard irrigation which consists of a daily water allotment delivered in one irrigation event. Cycles consist of water applications separated by rest intervals (time between irrigation events). Intermittent water applications decrease the time averaged application rate (TAAR), the volume of water applied divided by total time over which it is applied (Zur, 1976). Zur defined TAAR as a function of discharge rate, duration of application, and length of rest interval (1976). Warren and Bilderback (2005) suggest that TAAR is more important than the number of irrigation cycles in improving water retention.

Water Movement through Soils and Substrates

To understand how plant growth is affected by cyclic water applications, it must first be understood how water moves through a soil column or, in the case of container growing, the substrate in a container. Plants require that roots have access to water and oxygen during the growing season. Plants with insufficient access to soil water or oxygen can have reduced growth and eventually die; therefore it is desirable to have a balance between water drainage and retention so that roots have access to both water and oxygen.

For this reason, container substrates are often chosen for their water holding and drainage properties.

The physical characteristics of a substrate and container volume determine how much water is available for plant uptake (Spomer, 1982). Coarse media, like pine bark substrate, has low water holding capacity and high leaching potential (Fare et al., 1996). Secondary components of mixes are also important in determining the leaching potential of a substrate. Amending pine bark with highly porous material (low water holding capacity) rather than low porosity components (high water holding capacity) can increase leaching potential. Fare et al. (1996) reported that pine bark:sand mixes are more porous than pine bark:peat media.

Substrate composition also affects the pattern of water distribution in a substrate column as does the irrigation rate and rest interval, among other factors. During an irrigation event, water first occupies macropores, which offer little physical resistance. If the duration of the rest interval is sufficient, water will also fill micropores which are better at storing water for long periods of time than macropores. During the time between irrigation events, water will drain out of the macropores and progress down the soil column more easily than out of the micropores, in which water is under greater tension.

Proper manipulation of the timing and duration of irrigation applications can provide a consistent supply of water to roots. Initially, studies testing cyclic irrigation were performed on soil (Zur, 1976; Levin and Van Rooyen, 1977). Traditional irrigation regimes consisting of one continuous application per day can cause an inconsistent supply of moisture to plant roots and limit growth (Karam et al., 1994); however, cyclic irrigation of soils, if using optimal water volumes, application rates, and rest intervals, produces a continuously advancing wetting front, resulting in a consistent wetting pattern, thus providing consistent water to plant roots (Zur, 1976; Levin and Van Rooyen, 1977).

Lateral movement of water within a substrate column, which is affected by irrigation regimes, is essential for water retention within container substrates. Cyclic irrigation increases lateral movement of water into soil and decreases the volume lost past the root zone, compared to single applications (Levin and Van Rooyen, 1977; Levin and Van Rooyen, 1979). Furthermore, extremely dry pine bark media is resistant to rewetting, and irrigation of extremely dry containers often results in channeling of water through the substrate, resulting in increased leaching (Lamack and Niemiera, 1993).

Water Application Efficiency

Nurseries can conserve water by increasing the amount of applied water retained within the container, therefore reducing effluent. Water application efficiency (WAE) is a measure of how much applied water is retained within the rooting volume of a plant, and is expressed as:

$$[(\text{volume applied} - \text{volume leached}) / \text{volume applied}] \times 100$$

Cyclic irrigation has been shown to increase WAE in overhead, spray stake, and drip irrigation systems (Fare et al., 1994; Karam et al., 1994; Karam and Niemiera, 1994; Ruter, 1998; Tyler et al., 1996a). Adjusting irrigation rates, volumes and rest intervals can increase WAE, which generally increases with decreasing TAAR (Lamack and Niemiera, 1993). Warren and Bilderback (2005) recommend that a target TAAR of less than 10 mL/minute is needed to increase WAE over that of a single application. Irrigating cyclically results in an 11 to 38% increase in WAE of pine bark-based substrates

(Lamack and Niemiera, 1993; Ruter, 1998; Tyler et al., 1996a). WAE and duration of rest interval are directly related; however, if the rest interval is too long, pine bark substrate becomes hydrophobic and resistant to rewetting (Karam and Niemiera, 1994).

Leachate

The leaching fraction (LF) of a container is a measure of how much applied water is lost by the substrate and is calculated as:

[(volume leached / volume applied)] * 100

Low LFs reduce not only the volume of effluent, but also the amount of nutrients and chemicals leaving the container and possibly entering waterways. Cyclic irrigation can reduce leaching fractions regardless of the irrigation system being employed. By applying water in three cycles instead of one continuous application, volume of container leachate was reduced by 34% (Fare et al., 1994). Fare et al. (1996) reduced leachate volume by 54% when irrigating cyclically versus in one application, regardless of the length of time between cycles. Using pine bark:sand media and drip irrigation, cyclic irrigation regimes (2x, 3x or 6x) reduced volume of effluent with no effect on plant growth of *Cotoneaster* and *Rudbeckia* (Tyler et al., 1996a).

Some studies have demonstrated that higher quality plants can be produced with leaching fractions below the recommended rate; for example, *Ligustrum texanum* grown with 0.1 LF had higher foliar concentrations of nitrogen and phosphorus and higher quality ratings (Jarrell et al., 1983). Owen et al. (2008) found that reducing LF from 0.2 to 0.1 in a pine bark:sand media resulted in 64% reduction in leachate volume and a 64% reduction in effluent phosphorus concentration without adversely affecting plant dry weight of *Cotoneaster dammeri* Schneid. 'Skogholm'.

Time of irrigation applications also influences LF. Cyclic irrigation including afternoon applications resulted in decreased LF compared to cycles applied only in the morning hours (Warren and Bilderback, 2002); however, current Best Management Practices recommend that water be applied prior to 1000 hours when there is little drift from wind and low evaporative demand (Yeager et al., 2007).

Electrical Conductivity (EC)

Monitoring the pH and electrical conductivity (EC) of a substrate is important in managing irrigation and fertilization regimes of container-grown plants. While low LFs are desirable because they conserve water, the resulting solute accumulation can reduce plant growth and even be toxic. Past studies have demonstrated that the substrate EC levels of cyclically irrigated plants are higher than those of plants receiving irrigation in one cycle (Hicklenton and Cairns, 1995; Fain et al., 1999). Reducing LFs can reduce growth; for example, reducing LFs from 0.4 and 0.6 to 0 and 0.2 resulted in an 8% and 10% decrease in root and plant dry weight, respectively, of *Cotoneaster* grown in pine bark:sand media (Tyler et al., 1996b). This trade-off between water conservation and maximum growth indicates that EC management is essential when employing intermittent irrigation methods.

PH

The pH of a substance is a measure of its acidity and represents the concentration of hydrogen ions present. The responses of woody ornamental plants to substrate and water pH are dependent on species and cultivar as some are more tolerant of pH levels outside of the optimal range (5 to 6) (Bilderback, 2001) than others. Landis (1990) reported that the optimum pH level for organic substrates is 5.5, but values of 6 to 8 have been reported at a PIP nursery in Ohio (Zhu et al., 2005). Suboptimal pH levels can alter uptake of nutrients and reduce plant growth and quality, and the low buffering capacities of pine bark-peat substrates makes pH monitoring essential in container production (Mathers, 2007). A classic example is the substitution of hydrogen ions for calcium ions, an important component in stress signaling. Hydrogen ions can displace calcium ions from the exchange sites of a soil complex resulting in decreased calcium uptake, therefore reducing a plant's ability to adjust to environmental stress. Cyclic irrigation has not been shown to affect the pH of pine bark substrates (Fain et al., 1999).

Nutrient retention

Excessive irrigation decreases nutrient retention and efficacy (Tyler et al., 1996a; Tyler et al., 1996b). Nitrogen losses from controlled release fertilizers (CRFs) can reach 80% depending on the nutrient source, release mechanism, and irrigation method (Fare et al., 1994; Rathier and Frink, 1989). When nutrient retention increases, fewer nutrients are leached. In addition to reduced irrigation volumes, cyclic irrigation can be used to increase nutrient retention and decrease the amount of nutrients leaving a container. Decreases of 47 to 82% in N leached from containers have been recorded when using cyclic irrigation compared to a single application (Fare et al., 1996; Fain et al., 1999). The combination of decreased irrigation volume and increased cycle frequency can reduce the portion of N leached as NO3-N; for example, the percentage of applied N leached as NO3-N was 63% when 13 mm was applied in one cycle, but only 15% when 6 mm was applied in three cycles (Fare et al., 1994). Tyler et al. (1996a) suggested that in order to minimize NH_4-N leaching, the duration of the rest interval must be sufficient to recharge the cation exchange (CEC) of the substrate.

Growth response to water-conserving irrigation regimes

Function of water in plant growth

Water is an essential component in plant growth, constituting over 50% of tree fresh weight. Water uptake is essential for many plant functions including maintenance of leaf temperature and mineral uptake from soils. It is necessary for turgor maintenance and for cell enlargement, the latter being essential in leaf expansion and growth of tree rings (Kramer, 1987). Limiting water, or any component of the photosynthesis reaction, can inhibit carbon assimilation (A), and therefore growth.

Although not the only factor controlling plant productivity, substrate moisture is a variable which can be relatively easily monitored and manipulated to increase net photosynthesis and growth. Substrates maintained at or near 100% field capacity maximize growth in a variety of plants, including woody ornamental shrubs (Beeson, 1992). A 23% and 17% increase in shoot dry weight of *Acer rubrum* 'Franksred' trees occurred when irrigation was applied in three or six cycles, respectively, compared to one continuous application. This response was similar to that recorded by Timmer and Armstrong (1989) who observed maximum biomass production of *Pinus resinosa* seedlings when container capacity was maintained near 100%. Irrigation regimes supplying insufficient soil water can significantly reduce biomass; for example, substrate maintained at 73% and 57% container capacity irrigation regimes resulted in 21% and 43% reduction in biomass, respectively, compared to substrate maintained above 92% of container capacity (Timmer and Armstrong, 1989).

Height and Caliper response

Plant responses to cyclic irrigation include higher growth index, root growth, trunk diameter, shoot dry weight, height, and crop yield than plants receiving irrigation in only one cycle (Beeson and Haydu, 1995; Fain et al., 1999; Ismail et al., 2007; Keever and Cobb, 1985; Witmer, 2000). Tree caliper and height are of most concern to growers, because those are the standards by which landscape trees are sold (ANLA, 2004); therefore, an increase in tree caliper or height translates into more potential profit for the grower. Increased caliper and height growth of *Ulmus alata* Michx , *Acer rubrum* 'Franksred', *Acer rubrum* L. trees, and *Quercus acutissima* tree species and cultivars in response to cyclic irrigation has been reported; in some cases, caliper growth was 25% greater using cyclic irrigation compared to the once-daily irrigation (Beeson and Haydu, 1995; Fain et al., 1999; Witmer, 2000).

Growth responses to irrigation regimes can vary depending on the phase of the production cycle. Witmer (2000) monitored the response of PIP-produced *Acer rubrum* L. 'Franksred' trees to cyclic irrigation through a typical two-year production cycle. Cyclic irrigation (three times daily), as compared to one cycle, increased trunk diameter of *A. rubrum* trees only in the second year of production, whereas *Acer saccharum* Marsh trees responded similarly in both years of production. These results are consistent with the findings of Beeson and Haydu (1995), suggesting that cyclic irrigation may not be beneficial until roots fill a container, therefore making rapidly growing, large plants more responsive to cyclic irrigation than slow-growing, small plants (Beeson and Haydu, 1995; Witmer, 2000).

Biomass production and resource allocation

Plants must adjust to water limitations or growth will be reduced and death may occur (Lopez et al., 2009). Plant responses to short-term water stress include leaf senescence, growth reduction or cessation, temporary wilting and reduced photosynthesis, leaf temperature, and ET. Long-term water stress triggers drought tolerance mechanisms, resulting in increased root mass, leaf senescence, or osmotic adjustment whereby plants increase intracellular solute concentration (Chaves et al., 2003).

Responses of plants to water stress are evident not only in total plant mass but also in its distribution within a plant. Plants allocate biomass in an effort to optimize resource acquisition such that more mass will be devoted to the organ which is capable of obtaining the limiting resource; for example, water-stressed plants will increase mass allocation to below ground structures to obtain water and decrease allocation to above ground structures to reduce loss of water through stomata (Barbour, 1999). Trees growing on xeric sites have increased rooting volumes and depths than trees on mesic sites (Addington et al., 2006); however, most container volumes restrict the rooting space to less than 1% of that of naturally grown trees of similar size (Gilman and Beeson, 1996). Therefore, the ability of container-grown plants to increase root mass under limited water availability is somewhat restricted.

Water applications can be manipulated to manage biomass production and resource allocation. Plant growth can be manipulated by administering mild drought stress, which inhibits shoot elongation and keeps plants compact (Cameron et al., 2008). Conversely, regimes where water is not limiting increase shoot growth and decrease root:shoot ratios (Beeson and Haydu, 1995; Ruter, 1998). Cyclic irrigation applications decreased R:S of PIP-produced *Prunus* x *incamp* 'Okame' trees (Ruter, 1998), and *Ulmus alata* Michx. trees grown with cyclic irrigation (two or three cycles per day) had more shoot dry weight than trees receiving one continuous cycle per day (Beeson and Haydu, 1995).

Long-term plant growth can be expressed as a function of cumulative water stress using the water stress integral (Ψ_S). Used in ecological studies as a predictor of tree growth, Ψ_S is the cumulative measure of pre-dawn leaf water potential below a defined threshold (Linder et al., 1987; Myers, 1988). Reducing water stress at any point will reduce Ψ_S. Container-grown woody ornamental shrubs maintained at higher substrate moisture contents through cyclic irrigation had lower Ψ_S values than plants receiving one continuous pre-dawn application (Beeson, 1992). The difference in Ψ_S between cyclically irrigated plants and those receiving a single daily application occurred mainly between 1300 hr and 1600 hr indicating that cyclic irrigation reduces mid-day water stress. These findings are consistent with a study measuring sap flow in *A. rubrum* L. 'Franksred' trees, which demonstrated that afternoon irrigation applications alleviate mid-day water stress (Witmer, 2000).

The growth rate of woody ornamentals ultimately determines the length of their production cycle, and shorter production cycles typically increase profitability. Cyclic microirrigation has been shown to speed up the production process; for example, *Q.virginiana* Mill. trees micro-irrigated twice daily reached marketable size nearly three times faster than those irrigated overhead once daily, and *Acer rubrum* trees receiving three pulses of irrigation matured 1.82 times faster than similar trees receiving standard irrigation (Haydu and Beeson, 1997).

Irrigation Rate and Growth

Growth response

Daily water use (DWU), equivalent to evapotranspiration + leaching, is an increasingly popular parameter on which to base irrigation. Regulated deficit irrigation (RDI) is a water conserving method in which only a portion of DWU is applied. Numerous studies have demonstrated equal or superior growth rates of woody ornamental crops at reduced irrigation volumes (Groves et al., 1998; Martin et al., 1989; Roberts and Schnipke, 1987; Warsaw et al., 2009a; Warsaw et al., 2009b). Beeson and Haydu (1995) summarized that under cyclic irrigation the water volume required per area can be reduced by at least 25% and 50% for xeric or mesic species, respectively, with no effect on growth (compared to standard overhead irrigation). However, there may be a trade-off between water conservation and plant productivity if irrigation volume does not meet plant demand; in a study by Groves et al. (1998), a 40% reduction in applied water volume resulted in a 10% reduction of maximum growth.

Physiology of water stress

Basic water stress physiology

According to the soil-plant-atmosphere continuum (SPAC), water transport is a function of water potential (Ψ) of these factors and moves along a gradient of negative pressure. Reducing plant available water affects plants both directly, by limiting the amount of water available for photosynthesis and metabolism, and indirectly, by loss of turgor pressure of the guard cells which reduces stomatal conductance (g_S) and therefore uptake of CO_2.

At low to moderate water stress, a decrease in A is due to reduction in g_S (Allen et al., 2005a; Allen et al., 2005b, Chaves et al., 1991). As water becomes limiting, turgor pressure within guard cells is reduced, therefore decreasing stomatal aperture, and g_S. Decreases in substrate moisture have been shown to reduce A, g_S, and transpiration (E) in a number of species (Niu et al., 2008). Gas exchange rates affect the conversion of photosynthetically active radiation (PAR) into plant tissue (Allen et al., 2005b).

It has been hypothesized that cyclic irrigation increases growth by removing or reducing mid-day water limitations, thereby increasing the cumulative time of photosynthesis (Beeson, 1992; Witmer, 2000). Cyclic irrigation, by maintaining higher substrate moisture than one continuous application, delays or prevents stomatal closure, resulting in higher cumulative A (Bilderback, 2002). This response is usually observed in the afternoon when temperatures are highest. Warren and Bilderback (2002) found that afternoon irrigation applications were important in maintaining A rates during mid-day hours. At 1630 HR, plants receiving cyclic applications only in the morning (200, 400, 600 HR and 600, 900, 1200 HR) had A rates 86% less than those of plants receiving cyclic water applications in the afternoon (1200, 1500, 1800 HR) (Warren and Bilderback, 2002).

Water Use Efficiency

Up to 97% of water taken up by a plant is transpired through stomata (Taiz and Zeiger, 2006), and the initial response to limited water availability is stomatal closure (Hand et al., 1982; Medrano et al., 2002), which reduces CO_2 uptake and reduces assimilation. The relationship between transpiration and assimilation is the water use efficiency (WUE) and can be viewed as a measure of how efficiently a plant is using water to produce biomass (Anyia and Herzog, 2004). Increases in WUE have been shown when plant water availability is decreased (Sun et al., 1996). There are three methods commonly used to calculate WUE: instantaneous WUE (WUE_i), integrated WUE, and transpiration efficiency (TE).

Instantaneous WUE

Instantaneous WUE measures WUE at the leaf level, and is the ratio of the rate of CO_2 assimilation (measured at the leaf level) ratio to rate of water transpired. It is calculated using gas exchange data as:

A/E or A/g_s

Instantaneous WUE excludes water used in respiration and non-photosynthetic functions and is referred to as intrinsic WUE, because the response of E to changes in g_s and vapor pressure deficit (VPD) is inherent to a species (Bauerle et al., 2006; Bsoul et al., 2007; Cernusak et al., 2009). Reductions in transpirational losses without concomitant reductions in A can increase WUE_i and lead to substantial long-term increases in WUE (Yoo et al., 2009).

Integrated WUE

Carbon isotope discrimination (**Δ**), using the ratio of intercellular ^{13}C to ^{12}C, is used to calculate integrated water use efficiency, so-called because it provides a cumulative measure of plant status over the entire life of the plant. Under ideal conditions, plants discriminate against ^{13}C, the heavier isotope, during photosynthesis and preferentially incorporate ^{12}C, the lighter isotope, into biomass; however, as water stress increases, isotope discrimination decreases, and ^{13}C is more readily used, increasing the ^{13}C:^{12}C in plant tissue. Water stress and the subsequent stomatal closure result in increased ^{13}C:^{12}C of tissue relative to non water stressed plants, as intracellular ^{12}C is depleted and ^{13}C is used as a reactant in the photosynthesis (Cregg and Zhang, 2000).

The Δ calculation is based on the fact that the atmospheric concentration of $^{13}CO_2$ is a known constant, and the ^{13}C:^{12}C ratio of plant tissue is less than that of the atmosphere, indicating that plants discriminate against ^{13}C. This discrimination occurs in two stages in C3 plants. First, $^{13}CO_2$, the heavier isotope, does not diffuse through stomata as readily as $^{12}CO_2$. $^{13}CO_2$ diffuses at a rate of 4.4‰ that of $^{12}CO_2$. Secondly, fractionation occurs during carboxylation as Ribulose biphosphate carboxylase-oxygenase (Rubisco) has a greater affinity for $^{12}CO_2$ than $^{13}CO_2$ by a factor of 27‰ (Farquhar et al., 1989). Standardized Δ values are adjusted to PeeDee belemnite values and are expressed as δ (Craig, 1957). The response of integrated WUE to water stress is such that as water becomes limiting, Δ decreases and δ increases.

Transpiration Efficiency

Transpiration efficiency (TE) is the relationship between water absorbed by a plant and the total biomass produced and represents the whole plant water use efficiency. This method takes into account water used in other metabolic processes besides photosynthesis and is calculated as

[biomass produced / water used]

Water-limiting irrigation regimes have been used to increase the TE of a number of plants including *Capsicum annuum* L., *Catharanthus roseus*, and *Olea europaea* (Bacelar et al., 2007; Jaleel et al., 2008; Karam et al., 2009). In a study by Ismail et al. (2007), cyclic irrigation increased TE of tomatoes by 15%.

The response of WUE to drought stress is dependent on plant type, species, cultivar, tree age, provenance, and phenotype, and can be used as a screening process for selection of drought resistant plants (Adams and Kolb, 2004; Bacelar et al., 2007; Cernusak et al., 2009; Turnbull et al., 2002; Zhang and Cregg, 2005). In a study of mature forest trees, *Quercus rubra* L. trees had higher WUE values when grown on xeric sites than trees grown under mesic conditions, compared to *A. rubrum* L. trees which maintained a low WUE regardless of soil water availability, suggesting that *Q. rubra* minimizes transpirational water loss through stomatal regulation allowing for consistent rates of photosynthesis, whereas *A. rubrum* does not (Turnbull et al., 2002).

In an effort to gain insight into the functional morphology affecting the WUE response, models have been developed, which explain that coniferous evergreens are more resistant to drought stress (increased WUE) than broadleaf trees due to sunken stomata, lignified guard cell walls, and lower pre-dawn osmotic potential (an intrinsic measure of plant resistance to drought). The model also predicts that within coniferous species, *Pinus* species are more strongly affected by VPD than other species due to their low soil to leaf water conductance of xylem tracheids (Gao et al., 2002).

Specific Leaf Area

Plants also respond to stress through changes in leaf morphology. Specific leaf area (SLA) is the amount of light capturing surface per unit of biomass invested, and can be viewed as a plant's return on investment of a leaf (Milla et al., 2008). It is calculated as:

Leaf area / Leaf mass

Thicker, denser leaves have a lower SLA and thinner, less dense leaves have a higher SLA. Specific leaf area varies naturally between species, but is also a function of many environmental factors including temperature, ambient CO_2 concentration, leaf age, light intensity, and soil moisture (Milla et al., 2008). Decreases in SLA under water limiting conditions in plants have been documented in many broadleaf and evergreen trees including *Eucalyptus*, *Fagus*, and *Quercus suber* (Gouveia and Freitas, 2009; Milla et al., 2008; Myers and Landsberg, 1989).

Plant nutrient status

The relationship between SLA and photosynthetic response to leaf N can be expressed as a function of foliar N, such that at a given mass of foliar N, net assimilation (A_{max}) increases with rising SLA. Therefore, leaves that are thicker and/or denser will have a lower photosynthetic rate per unit foliar N resulting in lower A_{mass}, whereas leaves that are thinner and/or less dense will have a higher photosynthetic rate per unit leaf N and higher A_{mass} (Reich et al., 1998; Turnbull et al., 2002). This response is independent of environment or genotype (Reich et al., 1998). Response of SLA and N and A_{max} relationships are highest in forbs, followed by deciduous species, and lowest in evergreen species, regardless of leaf type or growth form (Reich et al., 1998).

The response of mass-based A to foliar N levels can also be viewed as a function of SLA, such that at a specific SLA, A_{max} increases with rising foliar N, resulting in higher A_{mass}. Furthermore, leaves with a lower SLA (thicker, denser leaves) have lower photosynthetic rates per unit of foliar N (flatter slopes), and therefore lower A_{mass}, whereas leaves with higher SLA have higher photosynthetic rates per unit of foliar N (higher slope).

Water is an important component of plant nutrient uptake for many reasons. Controlled release fertilizers, commonly used in container production of woody ornamental crops, are dependent on water for nutrient release (Fain et al., 2000). Movement of nutrients into roots is primarily a passive process, as nutrients are taken up in aqueous solution. Therefore, if the water volume is insufficient, nutrients will not be taken up, resulting in plant nutrient deficiencies; for example, foliar nutrient (N, P, K, Ca, Mg) concentrations declined with increasing moisture stress in *P. resinosa* seedlings (Timmer and Armstrong, 1989; Turnbull et al., 2002). Conversely, when excess water is applied, as is often done in container production, leaching of nutrients can occur, therefore reducing the quantities available for plant uptake. Reduced leachate volumes due to cyclic irrigation regimes increased foliar N concentrations compared to one continuous application (Karam et al., 1994), and foliar N can be an indicator of photosynthetic efficiency (Allen et al., 2005a;).

Nitrogen is an essential component of chlorophyll. The leaf greenness index, measured with a portable meter, was developed as a quick, easy, and nondestructive alternative to sample relative chlorophyll content. The handheld meter measures foliar absorbance of red and infrared wavelengths and calculates a value representative of the amount of chlorophyll in the leaf. Leaf greenness index readings have been correlated with chlorophyll and foliar nitrogen levels in ornamental and agronomic crops (Fritschi and Rey, 2007; Loh et al., 2002; Wang et al., 2004); however, some studies debate the validity of SPAD readings as a predictor of leaf N and chlorophyll content, citing leaf thickness, time, irradiance at time of measurement, and leaf water content as confounding factors (Marenco et al., 2009; Martinez and Guiamet, 2004; Nigam and Aruna, 2008). In a study of PIP-produced shade trees in the upper Midwest, leaf greenness index was correlated with assimilation rates and demonstrated a species specific correlation with foliar nitrogen (Klooster et al., 2010).

Chlorophyll fluorescence

Chlorophyll fluorescence can be used to test the efficiency of photosystems of a plant (Maxwell and Johnson, 2000). One aspect of chlorophyll fluorescence is Fv/Fm, which indicates the efficiency of PSII. Light energy absorbed by plants can be used for photosynthesis, dissipated as heat, or reradiated as light. These processes are competitive in that an increase in the quantity of light energy involved in one action dictates a decrease in the quantity used in the remaining two processes. Measuring the pulse of light (reradiation) from excited chlorophyll can garner information regarding photosynthesis and heat release (Maxwell and Johnson, 2000). Drought stress has been shown to decrease Fv/Fm (Brodribb and Holbrook, 2003); however, some studies (Havaux, 1992; Lu and Zhang, 1999) reported a highly drought stress-resistant PSII in species such as *Lycopersicon*, *Solanum* and *Tritium* species.

Exposure to a stress can confer resistance to other stresses (cross-resistance). Well-watered plants exposed to subsequent high temperatures have irreversible reductions in PSII efficiency compared to drought stressed plants (Havaux, 1992). This thermostability is thought to be part of a broader adaptation strategy, as water and temperature stress often occur simultaneously under field conditions.

Water potential

Water potential (Ψ) is a measure of the free energy of water and is the driving force that transports water from a substrate, through a plant, and into the atmosphere.

Water moves from high Ψ to low Ψ along a gradient of negative pressure. Under drought stress, Ψ values become increasingly negative, increasing the tension within the xylem under which the water is held. Stem water potential (Ψ_{Stem}) and leaf water potential (Ψ_{Leaf}) are the most common measurements of this type. Cavitation (embolism) occurs when tension falls rises above a species specific cavitation threshold, and the xylem fills with air (Tyree and Sperry, 1989). It has been shown that vascular plants close stomata to prevent xylem cavitation and the subsequent hydraulic conductance disfunction (Brodribb and Holbrook, 2003). Lethal leaf water potentials (Ψ_{lethal}) have been determined for some species including *Q. rubra* L (Ψ_{lethal} = -3.34 MPa) and *A. rubrum* L (Ψ_{lethal} = -4.43 MPa).

Leaf hydraulic conductance (K_{leaf}) is highly correlated with stomatal closure due to turgor loss and is very sensitive to Ψ. The ability to repair K_{leaf} following drought stress guides g_S recovery and is a measure of the vulnerability of a plant's hydraulic conductance. It has been suggested that ABA may also play a role in the recovery of g_S (Blackman et al., 2009). The ability to withstand increasing xylem tension may be an indication of a plant's ability to adapt to drought (Auge, 1997).

Mid-day Ψ is especially of interest in determining physiological responses to cyclic irrigation. Low mid-day Ψ can be caused by high transpiration rates of plants in well-watered media or by partially closed stomata in response to drought stress (Reich and Hinckley, 1989). On average, *Q. virginiana* Mill trees irrigated with multiple cycles experienced significantly lower Ψ_S values than trees irrigated only once daily, indicating reduced water stress (Beeson and Haydu, 1995).

Most of the water taken up by a plant is used in transpirational cooling, so as Ψ values decreases, so too does the plant's ability to dissipate heat. It has been suggested that the growth increase associated with cyclic irrigation is partially due to a decrease in mid-day water stress as elevated substrate moisture levels allow water transport to leaves, where latent heat can be absorbed, therefore decreasing the heat load (Beeson and Haydu, 1995). Keever and Cobb (1985) reported that applying pulses of water in the early afternoon, when temperatures are greatest, resulted in average decreases in daily canopy temperatures of 4.4°C in 'Hershey's Red' azalea (Rhododendron x 'Hershey's Red') . Furthermore, high root zone temperatures reduced g_S and Ψ and decreased root and shoot growth of *A. rubrum* trees (Graves et al., 1989).

Nursery growers in the upper Midwest are in need of scientifically based irrigation guidelines. A better understanding of the growth and physiological responses of PIP-grown trees to water-conserving irrigation regimes is needed to develop these guidelines. By applying traditional ecological sampling methods to horticultural studies, we can gain insight into the physiological mechanisms governing plant growth responses.

LITERATURE CITED

Adams, H. D. and T. E. Kolb. 2004. Drought responses of conifers in ecotone forests of northern Arizona: tree ring growth and leaf 13C. Oecologia 140: 217-225.

Addington, R.N., Donovan, L. A., Mitchell, R. J., Vose, J. M., Pecot, S. D., Jack, S. B., Hacke, U. G., Sperry, J. S., and R. Oren. 2006. Adjustments in hydraulic architecture of *Pinus palustris* maintain similar stomatal conductance in xeric and mesic habitats. Plant, Cell and Environment 29:535–545.

Adrian, J. L., C. C. Montgomery, et al. 1998. Cost Comparisons for Infield, Above Ground Container and Pot-in-Pot Production Systems. Journal of Environmental Horticulture 16(2): 65-68.

Allen, C. B., R. E. Will, and M.A. Jacobson. 2005a. Production efficiency and radiation use efficiency of four tree species receiving irrigation and fertilization. Forest Science 51(6): 556-569.

Allen, C. B., R. E. Will, R.C. McGarvey, D.R. Coyle, and M.D. Coleman. 2005b. Radiation-use efficiency and gas exchange responses to water and nutrient availability in irrigated and fertilized stands of sweetgum and sycamore. Tree Physiology 25(2): 191-200.

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

Anyia, A.O. and H. Herzog. 2004. Water use efficiency, leaf area, and leaf gas exchange of cowpeas under mid-season drought. Europ. J. Agronomy 20:327-339.

Auge, R.M., X. Duan, J.L. Croker, W.T. Witte, and C.D. Green. 1998. Foliar dehydration tolerance of twelve deciduous tree species. Journal of Experimental Botany 49(321) 753-759.

Bacci, L., Battista, P., and B. Rapi. 2008. An integrated method for irrigation scheduling of potted plants. Scientia Horticulturae 116:89-97.

Bacelar E.A., J.M. Moutinho-Pereira, B.C. Goncalves, H.F. Ferreira, and C.M. Correia. 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environmental and Experimental Botany 60:183–192.

Barbour, E. 1999. Terrestrial Plant Ecology - Third Edition, Benjamin-Cummings, Menlo Park, CA.

Bauerle, W. L., W. W. Inman, and J.B. Dudley. 2006. Leaf abscisic acid accumulation in response to substrate water content: Linking leaf gas exchange regulation with leaf abscisic acid concentration. Journal of the American Society for Horticultural Science 131(2): 295-301.

Beeson, Jr., R.C. 1992. Restricting overhead irrigation to dawn limits growth in container-grown woody ornamentals. Hortscience 27(9): 996-999.

Beeson, Jr., R.C. 1993. Relationship of potential evapotranspiration and actual evapotranspiration of Rhododendron sp. 'Formosa'. Proceedings of the Florida State Horticultural Society 106: 274-276.

Beeson, Jr., R.C. 2005. Modeling irrigation requirements for landscape ornamentals. Horttechnology 15(1): 18-22.

Beeson, Jr., R. C. 2006. Relationship of plant growth and actual evapotranspiration to irrigation frequency based on management allowed deficits for container nursery stock. Journal of the American Society for Horticultural Science 131(1): 140-148.

Beeson, Jr., R. C., M. A. Arnold, T.E. Bilderback, B. Bolusky, S. Chandler, H.M. Gramling, J.D. Lea-Cox, J. R. Harris P.J. Klinger, H.M. Mathers, J.M. Ruter, and T.H. Yeager. 2004. Strategic vision of container nursery irrigation in the next ten years. Journal of Environmental Horticulture 22(2): 113-115.

Beeson, Jr., R. C. and J. Haydu 1995. Cyclic microirrigation in container-grown landscape plants improves plant growth and water conservation. Journal of Environmental Horticulture 13(1): 6-11.

Bilderback, T.E. 2001. Environmentally compatible container plant production practices. Proc. Int. Symp. on Growing Media & Hydroponics. Acta Hort. 548. International Society of Horticultural Science.

Blackman, C. J., T. J. Brodribb, and G.J. Jordan. 2009. Leaf hydraulics and drought stress: response, recovery and survivorship in four woody temperate plant species. Plant, Cell and Environment 32(11): 1584-1595.

Brodribb, T. J. and N. M. Holbrook. 2003. Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. Plant Physiology 132(4): 2166-2173.

Bsoul, E., R. St Hilaire, and D.M. VanLeeuwen. 2007. Bigtooth maples from selected provenances effectively endure deficit irrigation. Hortscience 42(5): 1167-1173.

Cameron, R., R. Harrison-Murray, M. Fordham, S. Wilkinson, W. Davies, C. Atkinson, and M. Else. 2008. Regulated irrigation of woody ornamentals to improve plant quality and precondition against drought stress. Annals of Applied Biology 153: 49–61.

Cernusak, L. A., K. Winter, and B.L. Turner. 2009. Physiological and isotopic (delta13C and delta18O) responses of three tropical tree species to water and nutrient availability. Plant, Cell and Environment 32(10): 1441-1455.

Chaves, M. M. 1991. Effects of water deficits on carbon assimilation. Journal of Experimental Botany 42(234): 1-16.

Chaves, M. M., J. P. Maroco, and J.S. Pereira. 2003. Understanding plant responses to drought - from genes to the whole plant. Functional Plant Biology 30(3): 239-264.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica Et Cosmochimica Acta 12(1-2): 133-149.

Cregg, B. and J. Zhang (2000). Carbon isotope discrimination as a tool to screen for improved drought tolerance. Proceedings of the 11th Metropolitan Tree Improvement Alliance (METRIA) Conference, Gresham, OR.

Eakes, D. J., C.H. Gilliam, H.G. Ponder, W.B. Webster, C.E. Evans and C. Pounders. 1985. Influence of trickle irrigation on six field-grown woody landscape species based on net evaporation. Journal of Environmental Horticulture 3(3): 139-142.

Fain, G. B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1999. Cyclic irrigation improves irrigation application efficiency and growth of sawtooth oak. Journal of Arboriculture 25(4): 200-203.

Fain, G. B., C. H. Gilliam, K.M. Tilt, J.W. Olive, and B. Wallace. 2000. Survey of best management practices in container production nurseries. Journal of Environmental Horticulture 18(3): 142-144.

Fare, D. C., C.H. Gilliam, G.J. Keever, and R.B. Reed. 1996. Cyclic irrigation and media affect container leachate and ageratum growth. Journal of Environmental Horticulture 14(1): 17-21.

Fare, D. C., C. H. Gilliam, and G.J. Keever. 1992. Monitoring irrigation at container nurseries. HortTechnology 2(1): 75-78.

Fare, D. C., C. H. Gilliam, G.J. Keever, and J.W. Olive. 1994. Cyclic irrigation reduces container leachate nitrate-nitrogen concentration. Hortscience 29(12): 1514-1517.

Farquhar, G. D., R. Ehleringer, and K. T. Hubic. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Bio 40: 503-537.

Fitzpatrick, G. 1980. Water budget determinations for container-grown ornamental plants. Proc. Fla. State Hort. Soc. 93:166-168. 1980.

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

Gao, Q., P. Zhao, X. Zeng, X. Cai, and W. Shen. 2002. A model of stomatal conductance to quantify the relationship between leaf transpiration, microclimate and soil water stress. Plant, Cell and Environment 25: 1373-1381.

Gilman, E.F. and R.C. Beeson, Jr. 1996. Nursery production method affects root growth. Journal of Environmental Horticulture 14(2):88-91.

Gouveia, A.C. and H. Freitas. 2009. Modulation of leaf attributes and water use efficiency in Quercus suber along a rainfall gradient. Trees 23:267–275.

Graves, W. R., M. N. Dana, and R.J. Joly. 1989. Root-zone temperature affects water status and growth of red maple. Journal of the American Society for Horticultural Science 114(3): 406-410.

Groves, K. M., S. L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizers: I. Effect on plant growth and mineral nutrient content in containerized plant production. Journal of Environmental Horticulture 16(3): 176-181.

Hall, C.R., A.W. Hodges, and J.J. Haydu. 2005. Economic impacts of the green industry in the United States. 2 January 2009. <http://www.ufei.org/files/pubs/EconomicImpactsoftheUSGreen%20Industr%28NUCFACfinalreport%29.pdf>.

Hand, M. J., E. Young, and A.C. Vanconcelos. 1982. Leaf water potential, stomatal resistance, and photosynthetic response to water stress in peach seedlings. Plant Physiology 69: 1051-1054.

Havaux, M. 1992. Stress Tolerance of Photosystem 11 in Vivo Antagonistic Effects of Water, Heat, and Photoinhibition Stresses. Plant Physiol. 100:424-432.

Haydu, J. J. and R. C. Beeson, Jr., R.C. 1997. Economic feasibility of micro-irrigating container-grown landscape plants. Journal of Environmental Horticulture 15(1): 23-29.

Hicklenton, P. R. and K. G. Cairns. 1995. Plant water relations and mineral nutrition of containerized nursery plants in relation to irrigation method. Canadian Journal of Plant Science 76: 155-160.

Hight, A. and T. E. Bilderback. 1993. Substrate temperatures in above- and below-ground containers in a pot-in-pot system. Proceedings of the Southern Nursery Association Conference 39: 113-115.

Hill, R. W. and R. G. Allen. 1996. Simple irrigation scheduling calendars. Journal of Irrigation and Drainage Engineering-Asce 122(2): 107-111.

Ismail, S.M., K. Ozawa, and N.A. Khondaker. 2007. Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. First Power). Agricultural Water Management 95(2008): 116-122.

Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam, and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies 331(1): 42-47.

Jarrell, W.M., S. J. Whaley, and B. Miraftabi. 1983. Slow-release fertilizer and water management with container-grown *Ligustrum texanum*. Scientia Horticulturae 19(1-2): 177-190.

Jones, H. G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. Journal of Experimental Botany, Water-Saving Agriculture Special Issue 55(407): 2427-2436.

Karam, N.S., A.X. Niemiera, and C.E. Leda. 1994. Cyclic sprinkler irrigation and container substrate affects water distribution and marigold growth. Journal of Environmental Horticulture 12(4): 208-211.

Karam, N.S. and A.X. Niemiera. 1994. Cyclic sprinkler irrigation and pre-irrigation substrate water content affect water and N leaching from containers. Journal of Environmental Horticulture 12(4): 198-202.

Karam, F., R. Masaad, R. Bachour, C.Rhayem, and Y. Rouphael. 2009. Water and radiation use efficiencies in drip-irrigated pepper (*Capsicum annuum* L.): response to full and deficit irrigation regimes. European Journal of Horticultural Science 74(2): 79-85.

Keever, G. J. and G. S. Cobb. 1985. Irrigation scheduling effects on container media and canopy temperatures and growth of 'Hershey Red' azalea. Hortscience 20(5): 921-923.

Klooster, W.S., B.M. Cregg, R.T. Fernandez, and P. Nzokou. 2010. Growth and Photosynthetic response of pot-in-pot-grown conifers to substrate and controlled-release fertilizer. HortScience 45(1):1-7.

Knox, G. W. 1989. Water use and average growth index of five species of container grown woody landscape plants. Journal of Environmental Horticulture 7(4): 136-139.

Kramer, P. J. 1987. The role of water stress in tree growth. Journal of Arboriculture 13(2): 33-38.

Lamack, W.F. and A.X. Niemiera. 1993. Application method affects water application efficiency of spray stake-irrigated containers. Hortscience 28(6): 625-627.

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.

Levin, I. and F.C. Van Rooyen. 1977. Soil water flow and distribution in horizontal and vertical directions as influenced by intermittent water application. Soil Science 124(6): 355-365.

Levin, I., P.C. Van Rooyen, and F.C. Van Rooyen. 1979. The effect of discharge rate and intermittent water application by point source irrigation on the soil moisture distribution pattern. Soil Science Society of America Journal 43(1): 8-16.

Linder, S., M.L. Benson, B.J. Myers, and R.J. Raison. 1987. Canopy dynamics and growth of *Pinus radiata*. 1. Effects of irrigation and fertilization during a drought. Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere 17(10): 1157-1165.

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. Horttechnology 12(4): 682-686.

Lopez, R., J. Rodriguez-Calcerrada, and L. Gil. 2009. Physiological and morphological response to water deficit in seedlings of five provenances of *Pinus canariensis*: potential to detect variation in drought-tolerance. Trees 23: 509-519.

Marenco, R.A., S.A. Antezana-Vera, H.C.S. Nascimento. 2009. Relationship between specific leaf area, leaf thickness, leaf water content and *SPAD-502* readings in six Amazonian tree species. Photosynthetica 47(2):184-190.

Martin, C.A., H.G. Ponder, and C.H. Gilliam. 1989. Effects of irrigation rate and media on growth of *Acer rubrum* L. in containers. Journal of Environmental Horticulture 7(1): 38-40.

Martinez, D.E. and J.J. Guiamet. 2004. Distortion of the SPAD 502 chlorophyll meter readings by changes in irradiance and leaf water status. Agronomie 24(1): 41-46.

Mathers, H.M. 2007. Abiotic factors influencing root growth of woody nursery plants in containers. HortTechnology 17(2):151-162.

Maxwell, K. and G.N. Johnson. 2000. Chlorophyll fluorescence - a practical guide. Journal of Experimental Botany 51(345): 659-668.

MDA. 2010. Generally accepted agricultural and management practices for irrigation water use. Michigan Department of Agriculture. 15 February 2010. <http://michigan.gov/documents/MDA_Irrigation_GAAMP_129710_7.pdf>.

MDEQ. 2006. Water withdrawals for agricultural irrigation in Michigan. Michigan Department of Environmental Quality. 1 February 2010. < http://www.michigan.gov/documents/deq/deq-wd-wurp-Agriculture2006_208270_7.pdf>.

Medrano, H., J.M. Escalona, J. Bota, J. Gulias, and J. Flexas. 2002. Regulation of photosynthesis of C-3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Annals of Botany 89: 895-905.

Milla, R., P.B. Reich, U. Niinemets, and P. Castro-Diez. 2008. Environmental and developmental controls on specific leaf area are little modified by leaf allometry. Functional Ecology 22:565-576.

Monteith, J. L. 1964. Evaporation and environment. State and movement of water in living organisms. Symp. Soc. Expt. Biol. 19:205-234.

Myers, B.J. 1988. Water-stress integral - a link between short-term stress and long-term growth. Tree Physiology 4(4):315-323.

Myers, B.J. and J.J. Landsberg. 1989. Water stress and seedling growth of two eucalypt species from contrasting habitats. Tree Physiology 5:207-218.

NASS. 2007. Nursery crops summary 2006. National Agricultural Statistics Service and United States Department of Agriculture. 12 December 2010. <http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plans>.

Nigam, S.N. and R. Aruna. 2008. Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.). Euphytica 160:111-117.

Niu, G.H., D.S. Rodriguez, and W. Mackay. 2008. Growth and physiological responses to drought stress in four oleander clones. Journal of the American Society for Horticultural Science 133(2):188-196.

Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. Hortscience 43(3):906-912.

Penman, H.L. 1948. Natural evaporation from open water, bare soil and grass. Proc. Royal Soc. London A. 193(1032):120-146.

Rathier, T.M. and C.R. Frink 1989. Nitrate in runoff water from container grown juniper and Alberta spruce under different irrigation and nitrogen fertilization regimes. Journal of Environmental Horticulture 7(1):32-35.

Reich, P.B. and T.M. Hinckley. 1989. Influence of pre-dawn water potential and soil-to-leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. Functional Ecology 3:719-726.

Reich, P.B., D.S. Ellsworth, and M.B. Walters. 1998. Leaf structure (specific leaf area) modulates photosynthesis–nitrogen relations: evidence from within and across species and functional groups. Functional Ecology 12: 948–958.

Roberts, B.R. and V.M. Schnipke. 1987. The water requirements of five container-grown *Acer* species. Hortscience 22(5):1161-1161.

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

Ruter, J.M. 1998. Pot-In-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. Journal of Environmental Horticulture 16(3): 159-162.

Schoene, G.,T. Yeager, and D. Haman. 2006. Survey of container nursery irrigation practices in west-central Florida: An educational opportunity. Horttechnology 16(4): 682-685.

Schuch, U.K. and D.W. Burger. 1997. Water use and crop coefficients of woody ornamentals in containers. Journal of the American Society for Horticultural Science 122(5): 727-734.

Spomer, L.A. 1982. The effect of container soil volume on plant-growth. Hortscience 17(4): 680-681.

Sun, Z.J., N.J. Livingston, R. D. Guy, and G. J. Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce *(Picea glauca* (Moench) Voss) seedlings. Plant, Cell and Environment 19:887-894.

Taiz, L. and E. Zeiger. Plant Physiology, 4th edn. Sunderland, MA: Sinauer Associates Inc., 2006.

Thornthwaite, C.W. 1944. Report of the committee on transpiration and evaporation, 1943-44. Trans. Amer. Geophys. Union 25: 683-693

Timmer, V.R. and G. Armstrong. 1989. Growth and nutrition of containerized *Pinus resinosa* seedlings at varying moisture regimes. New Forests 3: 171-180.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F Schuster, K.J. Brown, V.C. Engel, and K.L. Griffin. 2002. Photosynthetic characteristics in canopies of *Quercus rubra*, *Quercus prinus* and *Acer rubrum* differ in response to soil water availability. Oecologia 130:515–524.

Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996a. Cyclic irrigation increases irrigation application efficiency and decreases ammonium losses. Journal of Environmental Horticulture 14(4): 194-198.

Tyler, H. H., S. L. Warren, and T.E. Bilderback. 1996b. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. Journal of Environmental Horticulture 14(4): 199-204.

Tyree, M.T. and J.S. Sperry. 1989. Vulnerability of xylem to cavitation and embolism. Annual Review of Plant Physiology and Plant Molecular Biology 40: 19-38.

Wang, Q.B., M.J. Chen, and Y.C. Li. 2004. Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. Journal of Plant Nutrition 27(3): 557-569.

Warren, S.L. and T.E. Bilderback. 2002. Timing of low pressure irrigation affects plant growth and water utilization efficiency. Journal of Environmental Horticulture 20(3): 184-188.

Warren, S.L. and T.E. Bilderback. 2005. More plant per gallon: Getting more out of your water. Horttechnology 15(1): 14-18.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009a. Water conservation, growth, and water use efficiency of container-grown woody ornamentals irrigated based on daily water use. Hortscience 44(5): 1308-1318.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009b. Container-grown ornamental plant growth and water runoff nutrient content and volume under four irrigation treatments. Hortscience 44(6): 1573-1580.

Weatherspoon, D.M. and C.C. Harrell. 1980. Evaluation of drip irrigation for container production of woody landscape plants. Hortscience 15(4): 488-489.

Witmer, R. 2000. Water use of landscape trees during pot-in-pot production and during establishment in the landscape. Horticulture. Blacksburg, Virginia Polytechnic Institute and State University. Doctor of Philosophy: 195.

Yeager, T., T. Bilderback, D. Fare, C. Gilliam, J. Lea-Cox, A. Niemiera, J. Ruter, K. Tilt, S. Warren, T. Whitwell, and R. Wright. 2007. Best management practices: Guide for producing nursery crops. 2nd ed. Southern Nursery Assn., Atlanta.

Yoo, C.Y., H.E. Pence, P.M. Hasegawa, and M.V. Mickelbart. 2009. Regulation of transpiration to improve crop water use. Critical Reviews in Plant Sciences 28: (6)410-431.

Zhang, J. and B.M Cregg. 2005. Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. Forest Ecology and Management 219:1-12.

Zhu, H., C.R. Krause, R.H. Zondag, R.D. Brazee, R.C. Derksen, M.E. Reding, and N.R. Fausey. 2005. A new system to monitor water and nutrient use in pot-in-pot nursery production systems. J. Environ. Hort. 23(1):47–53.

Zur, B. 1976. Pulsed irrigation principle for controlled soil wetting. Soil Science 122(5): 282-291.

CHAPTER TWO

GROWTH AND PHYSIOLOGY OF DECIDUOUS SHADE TREES GROWN UNDER CYCLIC IRRIGATION

Abstra

produc

regime

contai

Midw

Gledi

'Mor

assig

high

corre

rate.

grow

irrig

cycl

equa

carb

the r

meas

sensi

exch

Abstract

Efficient water management is essential for sustainable container nursery production. The objective of this study was to quantify the effects of cyclic irrigation regimes on the growth, water use efficiency (WUE), and physiology of common container-grown deciduous shade trees in Pot-in-Pot (PIP) production in the upper Midwest. Trees from seven taxa (*Acer freemanii* 'Jeffersred', *A. rubrum* 'Franksred', *Gleditsia triacanthos* 'Harve', *Gleditsia triacanthos* 'Skyline', *Quercus rubra*, *Ulmus* 'Morton', and *Ulmus* 'Morton Glossy'), grown in #25 containers, were randomly assigned to irrigation regimes with varying combinations of irrigation rates (low and high) and daily cycle frequencies (one or four). Low and high irrigation rates corresponded to 1 cm and 2 cm depths, respectively. Applications of the low irrigation rate, regardless of the number of cycles in which it was applied, reduced stem radial growth of all taxa except *A. freemanii* and *G. triacanthos* 'Harve' compared to the high irrigation rate. Physiological parameters were more responsive to irrigation rate than cycle frequency. Mid-day leaf stomatal conductance (g_S) of trees irrigated cyclically was equal to or less than trees irrigated once daily. Increasing cycle frequency only increased carbon isotope discrimination (Δ) of foliar and wood tissue in *A. rubrum* trees. Overall, the response of instantaneous water use efficiency (WUE_i) derived from gas exchange measurements to irrigation treatment was inconsistent. Δ of plant tissue is a more sensitive indicator of plant water stress compared to WUE_i derived from leaf gas exchange measurements.

Introduction

Water management is a growing concern for nursery producers because of a predicted decrease in water availability, anticipated increase in water costs, and stricter water use regulations (Beeson et al., 2004). Growers commonly overwater, applying a fixed rate of irrigation exceeding plant water use, which may result in excessive water withdrawals. Container growing also required the addition of fertilizer for optimum crop growth. The resulting, and often highly concentrated, container solution is highly susceptible to leaching, due to the low cation exchange capacity of commonly used pine-bark based substrates (Bilderback, 2001a). The development of irrigation programs which conserve water without sacrificing tree growth and quality will allow the nursery industry to reduce input costs and adapt to future water use regulations and decreased water availability.

Plant responses to cyclic irrigation, a method by which fractions of a plant's daily water allowance is applied in multiple cycles, include higher growth index, root growth, trunk diameter, shoot dry weight, height, and crop yield than plants receiving irrigation in only one cycle (Beeson and Haydu, 1995; Fain et al., 1999; Ismail et al., 2007; Keever and Cobb, 1985; Ruter, 1998; Witmer, 2000). In some cases, caliper growth was 25% greater using cyclic irrigation compared to the traditional method of applying a single cycle in the morning (Beeson and Haydu, 1995; Fain et al., 1999; Witmer, 2000).

Equal or superior growth rates of woody ornamental crops at reduced irrigation volumes compared to commonly applied irrigation rates have also been documented compared to commonly applied irrigation rates (Groves et al., 1998; Martin et al., 1989; Roberts and Schnipke, 1987; Warsaw et al., 2009a; Warsaw et al., 2009b). Beeson and

Ha

are

wi

be

do

Z

(H

as

re

o

(

P

i

2

(

Haydu (1995) summarized that under cyclic irrigation, the water volume required per area can be reduced by at least 25% and 50% for xeric or mesic species, respectively, with no effect on growth compared to standard overhead irrigation. However, there may be a trade-off between water conservation and plant productivity if irrigation volume does not meet plant demand (Groves et al., 1998).

Up to 97% of water taken up by a plant is transpired through stomata (Taiz and Zeiger, 2006), and the initial response to limited water availability is stomatal closure (Hand et al., 1982; Medrano et al., 2002), which prohibits CO_2 uptake and reduces assimilation (A). It has been hypothesized that cyclic irrigation increases growth by removing or reducing mid-day water limitations, thereby increasing the cumulative time of photosynthesis (Beeson, 1992; Witmer, 2000).

The relationship between transpiration and assimilation is the water use efficiency (WUE) and can be viewed as a measure of how efficiently a plant is using water to produce biomass (Anyia and Herzog, 2004). Several studies have demonstrated increases in WUE when trees are exposed to water-limiting irrigation regimes (Anyia and Herzog, 2004; Ningbo et al., 2009; Warren and Bilderback, 2002). There are three methods commonly used to calculate WUE: instantaneous WUE (WUE_i), integrated WUE, and transpiration efficiency.

Instantaneous WUE measures WUE at the leaf level, and is the ratio of the rate of CO_2 assimilation (measured at the leaf level) ratio to the rate of water transpired (E) or the rate of CO_2 uptake (g_s). It is calculated using gas exchange data as:

A/E or A/g_s

Inst

fun

and

20

inc

en

13

pr

di

in

is

th

w

p

s

2

t

g

v

b

d

Instantaneous WUE excludes carbon losses due to respiration and non-photosynthetic functions and is referred to as intrinsic WUE, because the response of E to changes in g_S and VPD is inherent to a species (Bauerle et al., 2006; Bsoul et al., 2007; Cernusak et al., 2009). Reductions in transpirational losses without concomitant reductions in A can increase WUE_i and lead to substantial long-term increases in WUE (Yoo et al., 2009).

Stable carbon isotope analysis has become an important method of assessing environmental stress. Carbon isotope discrimination (Δ), using the ratio of intercellular ^{13}C to ^{12}C, is used to calculate integrated water use efficiency, so-called because it provides a cumulative measure of plant status over the time tissues were formed. Plants discriminate against ^{13}C, the heavier isotope, during photosynthesis and preferentially incorporate ^{12}C, the lighter isotope, into biomass. As water stress increases however, isotope discrimination decreases, increasing the ^{13}C:^{12}C in plant tissue. Water stress and the subsequent stomatal closure result in increased ^{13}C:^{12}C of tissue relative to non-water-stressed plants, as intracellular ^{12}C is depleted and ^{13}C is used as a reactant in photosynthesis (Cregg and Zhang, 2000).

Ecological studies have documented decreases in Δ of deciduous shade tree species in response to limited water availability (Gouveia and Freitas, 2009; Keitel et al., 2006; Merchant et al., 2010); however, these studies have only included field-grown trees. By definition, Δ of plants grown under ambient CO_2 concentrations is a product of g_S and ^{13}C: ^{12}C (δ) fixed by Rubisco (carboxylation) and can be used as a surrogate for WUE_i (Cregg and Zhang, 2000; Seibt et al., 2008). Δ is inversely related to WUE_i (A/g_S), because as A increases or g_S decreases, C_i decreases, therefore increasing δ and decreasing Δ (Cregg and Zhang, 2000).

Transpiration efficiency (TE) is the relationship between water used by a plant and the total biomass produced and represents the whole plant water use efficiency. This method takes into account water used in other metabolic processes besides photosynthesis and is calculated as

[biomass produced / water used]

Water-limiting irrigation regimes have been used to increase the TE of a number of plants including *Capsicum annuum* L., *Catharanthus roseus*, and *Olea europaea* (Bacelar et al., 2007; Jaleel et al., 2008; Karam et al., 2009). Cyclic irrigation has been used to increase the TE of tomatoes by 15% compared to single applications (Ismail et al., 2007).

This study is part of an overall program to improve nursery production of potential ash tree replacement species in the upper Midwest. It was designed to test the effects of cyclic irrigation regimes on PIP-grown deciduous shade trees in Northern temperate climates. The main goal was to develop irrigation guidelines for growers in the upper Midwest that conserve water and reduce leaching of nutrients without sacrificing crop growth. The objectives of this study were to 1) determine effects of cyclic irrigation programs on tree growth, 2) explore underlying physiological mechanisms, and 3) examine the fate of nutrients in response to cyclic irrigation treatments. We also sought to compare various methods of WUE measurements.

Materials and Methods

Site description and experimental design

This study was conducted at the Sandhill site at the Michigan State University Horticulture Teaching and Research Center (HTRC) (lat.: 42.6734°N, long.: 84.4870°E, elev.: 264 m) in Holt, Michigan. Trees were grown using a pot-in-pot (PIP) system. The soil

spac

Rim

was

Pla

In

(J.

(Gl

use

'Fr

'Sl

C.

Sc

an

fa

Ir

T

ir

C

w

co

at

soil on site was a well-drained loamy sand (83.1% sand, 8.7% silt, 9.3% clay). Tree spacing was 1.4 m and 1.8 m on-center within rows and between rows with rows offset. Rims of the socket pots were approximately 2.5 cm above the ground, and the ground was covered with landscape fabric to control weeds.

Plant materials

In April 2008, one-hundred-and-sixty 25 to 37 cm (1" to 1-½") diameter bare-root liners (J. Frank Schmidt and Son Co., Boring, OR) were planted in #25 (104.4 L) containers (GL10000, Nursery Supplies, Inc., Chambersburg, PA). Sixteen trees of each taxa were used: *Acer ×freemanii* E. Murray (*rubrum* × *saccharinum*) 'Jeffersred', *A. rubrum* L. 'Franksred', *Gleditsia triacanthos* 'Harve', *Gleditsia triacanthos* L. var. inermis (L.) Zab 'Skyline', *Quercus rubra* L., *Ulmus. pumila* L. × *japonica* (Rehder) Sarg. × *wilsoniana* C.K. Schneid. 'Morton Glossy', and *Ulmus japonica* (Rehder) Sarg. *x wilsoniana* C.K. Schneid. 'Morton'. Trees were planted in an 80:20 (volume:volume) mix of pine bark and peat moss (Renewed Earth, Inc., Kalamazoo, MI) and installed in MSU's PIP facility. Container capacity of the media was 44.5%.

Irrigation treatments

Four trees of each taxa were randomly assigned to one of four irrigation regimes. Treatments consisted of combinations of daily irrigation volume and number of daily irrigation cycles (one or four). The once-daily irrigation event occurred at 0600 h daily. Cyclic irrigation treatments, delivering 25% of the daily water allotment in each cycle, were applied at 0600, 1000, 1400, and 1800 hr daily. Low and high irrigation rates corresponded to 1 cm and 2 cm depths, respectively. Low and high volumes were applied at a rate of 9.5 L/min. and 18.9 L/min., respectively. Daily irrigation depths were based on daily water use of a container-grown conifer in Michigan documented by Warsaw et al. (2009a). Average leaching fraction, sampled from *Ulmus* 'Morton Glossy' trees on three dates in 2009 (1 August, 6 August, and 14 August), was zero.

Irrigation was controlled by two timers (Nelson 8014 series Solo Rain); one timer ran once daily and the other ran four times per day. Irrigation volume was controlled via selection of emitter. During the first half of the 2008 growing season, water was delivered via non pressure-compensating spray stakes. Due to low distribution uniformity, the system was retrofitted with pressure-compensating drip emitters (Netafim, Fresno CA) in July 2008. In July 2009, the system was retrofitted with PCNL pressure-compensating spray stakes (Netafim, Fresno CA) to increase distribution uniformity within pots.

The experimental design was a strip-split plot design with number of cycles as the main plot factor, species as the subplot factor and irrigation rate as the sub-subplot factor. All combinations of species x irrigation rate x cycle frequency were replicated four times, and trees were arranged in the PIP system to allow for blocking in time of physiological measurements. One row on each side of the plot served as a guard row.

Trees were top-dressed with 400 g of 15-9-12 Osmocote® Plus 8-9 month release (Scotts, Inc.) in the spring of 2008 and 2009. Weeds were controlled as needed through hand-weeding. Minimal pruning was performed throughout both seasons as needed. Growing pots were lifted from the socket pots and turned periodically throughout the 2008 season to prevent rooting out. In June 2009, air and water permeable membranes impregnated with copper sulfate (SpinOut, Texel Agrotextiles, Quebec, Canada) were placed between the growing pot and the socket pot to prevent rooting out. In 2008 and

2009 respectively, irrigation was applied from 3 June to 1 October and from 21 May to 1 October.

Growth

Tree growth was measured monthly from May to October. Growth measurements included tree height, trunk caliper, crown spread, and light interception. Height was measured using a standard height pole placed perpendicular to the rim on the north side of the container. Height was measured to the highest live point of the tree. Average trunk caliper was assessed using two measurements perpendicular to each other, north-south and east-west, at a height 7.5 cm above the bud union. Crown spread was measured in two directions perpendicular to each other, north-south, and east-west, to the tips of the longest live branches, and a geometric average was calculated.

Gas exchange

Leaf gas exchange was measured periodically in each growing season using a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE). Due to their compound leaf structure, *Gleditsia* species were not included in leaf gas exchange sampling. One fully-expanded, sun-exposed leaf on each tree was sampled using a 3x2 cm leaf chamber equipped with a red/blue light-emitting diode light source (Li-6400-02B, Li-Cor) providing a quantum flux of 1500 $\mu mol \cdot m^{-2} s^{-1}$. Light saturated assimilation (A_{max}; $\mu mol \cdot m^{-2} \cdot s^{-1}$) and stomatal conductance to water vapor (g_S) were measured using optimum conditions within the chamber: CO_2 concentration at 400 $\mu mol \cdot mol^{-1}$ and flow of air at 500 $\mu mol \cdot s^{-1}$. To minimize temperature effects during sampling, the block temperature of the Li-6400 was set at the estimated high temperature for the day.

Measurements were recorded after the readings had stabilized on the system's real-time graphics screen. Intrinsic water use efficiency (WUE_i) was calculated as: A_{max} / g_s.

Chlorophyll fluorescence

The ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) was measured on trees of all species (excluding *Gleditsia* species) using an OS-30p chlorophyll fluorometer (Opti-Sciences, Hudson, NH). On 12 August 2009, three randomly selected, mature sun-lit leaves on each tree were harvested, and a cuvette provided by the manufacturer was attached to the interveinal portion of each leaf. Leaves were allowed to dark acclimate for 10 minutes before a reading was taken.

Chlorophyll content

Relative chlorophyll content was assessed using a Minolta SPAD 502 chlorophyll meter (Spectrum Technologies Inc., Plainfield, IL). The handheld device was clamped on to a randomly selected, fully expanded leaf and the value recorded. This process was replicated to obtain five values for each tree.

Carbon isotope discrimination

Leaves from all trees except *Gleditsia* species were collected on 23 September 2009 and dried to a constant weight. At the end of the 2009 season, stem tissue was harvested 7.5 cm above the graft union using an increment corer and dried to a constant weight. In cases where rings were not visible, cores were dyed with stain to improve ring identification. If rings were still not distinguishable, trees were destructively harvested and a 1.25 cm sample of the trunk removed and sanded to improve ring identification. Rings were separated by year and 1-2 mg of tissue spanning the width of the ring was removed. Foliar tissue was ground using a coffee grinder and passed through a #40 screen. Foliar (2-3 mg) and wood (1-2 mg) tissue was packed into 5x9 mm tin capsules and placed in a 96-well sample tray. Samples were sent to the University of California at Davis Stable Isotope Facility for analysis of the relative abundance of ^{13}C and ^{12}C. The resulting Δ values were expressed relative to international standards PeeDee Belemnite (limestone) (Craig, 1957).

Diurnal water potential

Leaf water potential (Ψ) of all trees within two taxa (*Q. rubra* and *Acer* species) was measured on two dates during the growing season; once on a day with relatively low VPD and once on a day with relatively high VPD. We selected *Q. rubra* and *Acer* trees for sampling, because they have contrasting vessel arrangements and hydraulic architecture; *Quercus* trees have a ring porous vessel arrangement, while *Acer* trees have a diffuse porous arrangement. *A. freemani* was sampled on 21 and 26 August 2008 and 13 August 2009, and *A. rubrum* was sampled on 22 June 2009. Times of sampling corresponded with gas exchange measurements. At two hour intervals, from pre-dawn to 2000 hours, one fully expanded leaf per tree was measured for gas exchange then harvested. Leaves were stored in an airtight plastic bag in a cooler of ice until the leaf water potential (Ψ) was determined using a PMS pressure chamber (PMS, Albany, OR). Dates of sampling were 25 June and 18 July in 2008 and 13 June and 13 August 2009. On 13 August 2009, a standard chamber using ambient environmental conditions was used for gas exchange measurements.

Specific leaf area and foliar analysis

All trees except *Gleditsia* species were sampled for specific leaf area (SLA). A random subsample of 20 fully expanded, sun-lit leaves from each tree was harvested in

July 2009. Leaves were scanned with a leaf area meter (LI-3000, Li-Cor), then oven-dried to a constant weight. Specific leaf area was calculated as: leaf weight (g) / leaf area (g). Leaves were harvested from all species excluding *Gleditsia* on 27 July, 2009, dried, and sent to a commercial lab for foliar nutrient analysis (Scotts Inc., Lincoln, NE).

Substrate moisture measurements

Sixteen trees spanning two blocks and representing each species and treatment were monitored throughout the 2009 season for substrate moisture and temperature. One 30-cm long moisture probe (CS616, Campbell Scientific) measuring volumetric water content (VWC) was installed in each pot approximately 10 cm from the edge of the container at a 45° angle. Probes were connected to a CR1000 datalogger (Campbell Scientific) and measured temperature every 30 seconds while recording 15-minute averages. Maximum, minimum, and average temperatures along with time of maximum and time of minimum were recorded every 1 hour. Substrate VWC values were calibrated for organic substrates according to manufacturer's standards. Two electrical rain gauge transmitters (Texas Electronics, Inc., Dallas, TX) were installed to estimate precipitation and connected to the datalogger via the multiplexer.

Nitrate-N, pH, and electrical conductivity in leachate

Leachate was collected from a subsample of three species using the pour-through procedure (Wright et al., 1986; Bilderback, 2001b). *Acer freemani*, *Ulmus* 'Morton Glossy', and *G. triacanthos* 'Skyline' (16 of each) were sampled in July 2008 and in May and July 2009. Growing pots were removed from socket pots, then media was saturated and allowed to drain for 30 minutes. Four liters of water were then applied to each tree, and leachate was collected. Electrical conductivity (EC; Oakton Con110 series, Eutech

Instruments, Vernon Hills, IL) and pH (AB15 Accumet basic, Fisher Scientific, Pittsburgh, PA) were measured in the laboratory after collection. Nitrate-N analysis was performed by the MSU Soil and Plant Nutrient Laboratory using flow injection with cadmium reduction (Huffman and Barbarick, 1981). Irrigation water, which was sampled at each collection time, averaged: N03-N, 0.24 ppm; EC, 0.8 dS/M; and pH, 7.8.

Statistical analysis

Data were analyzed using SAS version 9.1 software (SAS Institute Inc., Cary, NC). All variables were tested for normality using PROC UNIVARIATE. PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. Mean separation was performed using Tukey's adjustment. The effects of sampling time for gas exchange, WUE_i, Ψ, SPAD readings, foliar chlorophyll content (CC), and leachate NO3-N, pH, and EC data were analyzed using repeated measures within PROC MIXED. Mid-day g_S and leachate NO3-N was analyzed using a log transformation, and WUE_i was analyzed using a square-root transformation. Pearson correlation coefficients for growth and physiological parameters were determined using PROC CORR.

Results

Weather

Mean maximum daily air temperatures during the growing seasons (1 May to 1 October) were 25.0°C and 23.8°C for 2008 and 2009, respectively. Total precipitation amounts during that time were 462 mm in 2008 and 425 mm in 2009. Total (1 May to 1 October) reference evapotranspiration (ET_0) during the growing seasons exceeded rainfall by 308 mm and 244 mm in 2008 and 2009, respectively (Fig. 2.1). The average minimum and maximum air temperatures during the winter months (1 December 2008 to

31 March 2009), as reported by the Michigan Automated Weather Network (MAWN), were -7.9°C and 2.0°C (2010).

Substrate moisture

High irrigation rates applied cyclically resulted in greater increases in substrate moisture than low rates applied cyclically. Irrigating cyclically decreased the amplitude of daily fluctuations in substrate moisture content. Within the season, substrate moisture fluctuations varied greatly (Fig. 2.2).

Water use

Daily water use was estimated from volumetric moisture content data of four species: *A. rubrum*, *G. triacanthos* 'Harve', *Q. rubra*, and *Ulmus* 'Morton' for the 2009 growing season. Average daily water use over the course of the growing season was highest in *Ulmus* 'Morton', followed by *A. rubrum*, *Q. rubra*, and *G. triacanthos* 'Harve'. Total 2009 seasonal water use of *Ulmus* 'Morton' was more than 75% that of *G. triacanthos* 'Harve'. Crop coefficients (K_C) peaked in September. On average, K_C values were highest in *Ulmus* 'Morton', followed by *A. rubrum*, *G. triacanthos* 'Harve', and *Q. rubra* (data not shown).

Growth responses

Caliper and height

Across species, tree growth was more responsive to irrigation rate than number of irrigation cycles per day (Table 2.1). A strong species response was observed in most growth and physiological responses. The high irrigation rate increased stem radial growth relative to the low rate in all taxa except *A. freemanii* and *G. triacanthos* 'Harve' (Fig. 2.3) but increased ($p \leq 0.05$) height growth only in *Ulmus* 'Morton' trees. The low rate reduced ($p \leq 0.05$) crown growth in *Ulmus* species compared to the high rate. Most of the reduction in growth caused by the low irrigation rate occurred in the second year of the study (data not shown).

Gas exchange

Mid-day and diurnal A_{max} and g_s responses were affected by sampling date and taxa (Table 2.2), therefore samples were analyzed separately for each taxa and date. There was no clear trend in gas exchange responses to irrigation treatment. Mean mid-day A_{max} and g_s values were lowest in *Acer* and *Q. rubrum* trees, and highest in *Ulmus* trees (Table 2.3). Mean mid-day A_{max} values were correlated with total caliper growth of *A. freemanii* ($r=0.21$, $p \leq 0.05$), *Q. rubra* ($r=0.26$, $p \leq 0.01$), and *Ulmus* 'Morton' ($r=0.16$, $p \leq 0.05$)trees. Irrigation rate affected gas exchange in trees of all taxa on at least one sampling date. Increasing the irrigation rate increased ($p \leq 0.05$) g_s and A_{max} of *Q. rubra*, *A. freemanii*, and *Ulmus* 'Morton' trees on one sampling date (data not shown).

The only effect of cycle frequency on mid-day gas exchange occurred late in the 2009 season in *A. rubrum* trees and *Ulmus* 'Morton Glossy' trees. In both instances, trees receiving four cycles had lower ($p \leq 0.05$) g_s than trees receiving only one cycle (Fig. 2.4); however, on one sampling date, *Ulmus* 'Morton Glossy' trees receiving the low rate in one cycle had lower ($p \leq 0.05$) A_{max} values than *Ulmus* 'Morton Glossy' trees in all other treatments (Fig. 2.5).

Irrigation treatment did not affect diurnal gas exchange of *Q. rubra* trees. Diurnal courses of gas exchange of *Acer* trees was affected by irrigation treatment on all days sampled, and the response occurred mid-day (1200 hr to 1800 hr). The low irrigation rate reduced ($p \leq 0.01$) g_s and A_{max} of *A. rubrum* trees at 1200 hr and 1600 hr, respectively on 26

August 2008 (Fig. 2.5). A_{max} and g_s of *A. freemanii* trees was reduced ($p \leq 0.05$) at 1600 hr on one date (21 August 2008) when trees were irrigated with the low rate applied in one cycle compared to the high rate in four cycles (Fig. 2.6). A_{max} values of *A. freemanii* trees were reduced ($p \leq 0.05$) at 1400 hr on one date (26 August 2008) when trees were irrigated with the low rate applied in four cycles compared to the high rate in four cycles. The effect of cycle frequency was less evident than the effect of irrigation rate. Increasing cycle frequency increased ($p \leq 0.05$) A_{max} values of *A. freemanii* trees on only one date (21 August 2008) at 1400 hr.

Diurnal water potential

Diurnal Ψ responses to irrigation regime were more pronounced than those of gas exchange and occurred most often in the afternoon hours. Values of Ψ of *Acer* trees were higher throughout the day than those of *Q. rubra* trees in 2008 but were similar in 2009. *Acer* trees were also more responsive to irrigation regime than *Q. rubra* trees. There was only one date (23 June 2009) on which Ψ of *Q. rubra* trees was affected by irrigation treatment; at 1200 hr and 1600 hr, the low irrigation rate reduced ($p \leq 0.05$) Ψ compared to trees irrigated with the high rate (Fig. 2.7). The response of Ψ of *Acer* trees to irrigation treatment was inconsistent (Fig. 2.8). The low irrigation rate reduced ($p \leq 0.05$) Ψ values of *Acer* trees in the afternoon hours (1400 hr and 1800 hr) on only one (13 August 2009) of the four dates sampled (data not shown). Within cyclic irrigation treatments, *Acer* trees receiving the high rate had lower ($p \leq 0.05$) Ψ values than *Acer* trees receiving the low rate on two dates. Pre-dawn water potential values were similar regardless of irrigation treatments.

WUE

Instantaneous WUE

Mid-day WUE_i values calculated from mid-day gas exchange measurements were affected by sampling date and taxa, therefore samples were analyzed separately for each taxa and date. On average, WUE_i values were higher in year one than year two (data not shown). Mean WUE_i values varied by taxa (Table 2.3). Significant responses of WUE_i of trees to irrigation treatments were rare and inconsistent, and all occurred in the second year of the study. Irrigation rate did not affect WUE_i of any species on any date. Values of WUE_i of *A. freemanii*, *Q. rubra*, and *Ulmus* 'Morton' trees were unresponsive to irrigation treatment.

Cycle frequency affected WUE_i of only *A. rubrum* and *Ulmus* 'Morton Glossy' trees. *Acer rubrum* trees receiving the low rate applied in one cycle had higher ($p \leq 0.05$) WUE_i than other trees of that species receiving any other treatment on 5 June 2009. Increasing cycle frequency increased ($p \leq 0.05$) WUE_i of *A. rubrum* on one date (31 July 2009) and *Ulmus* 'Morton Glossy' trees on one date (4 September 2009).

Integrated water use efficiency

Isotopic composition of wood tissue varied by species but was unaffected by year; therefore, year one and year two samples were pooled together for further analysis. Foliar Δ values were correlated with Δ values of 2009 wood tissue only in *Q. rubra* ($r=0.75$, $p \leq 0.0001$), *Ulmus* 'Morton' ($r=0.59$, $p \leq 0.0001$), and *A. freemanii* ($r=0.19$, $p \leq 0.05$) trees. *Quercus rubra* and *A. rubrum* trees had the lowest Δ values in both foliar and wood tissue.

Irrigation rate affected the foliar isotopic composition of only *Acer* and *Q. rubra* trees. The low rate increased Δ of *Q. rubra* ($p \leq 0.01$) and *Ulmus* 'Morton' ($p \leq 0.05$)

foliage compared to the high rate (Fig. 2.9). *Acer rubrum* trees irrigated with the low rate in one cycle had lower ($p \leq 0.05$) foliage Δ values than all other treatments (Fig. 2.9); however, irrigating with only one cycle increased Δ values of wood tissue, regardless of the application rate (Fig. 2.10). Analysis of wood tissue yielded significant responses to irrigation rate only of *Ulmus* trees (Fig. 2.11). Foliar Δ was correlated with WUE_i only in *Ulmus* 'Morton' ($r=-0.17$, $p \leq 0.05$) and Ulmus 'Morton Glossy' ($r=-0.18$, $p \leq 0.05$). Δ of wood tissue was not correlated with WUE_i in any taxa. Leaf Δ was correlated with mid-day leaf g_S in *Ulmus* 'Morton Glossy' ($r=-0.27$, $p \leq 0.01$), *A. freemanii* ($r=-0.21$, $p \leq 0.01$), and *Q. rubra* ($r=-0.22$, $p \leq 0.05$) trees.

Leaf structure

Specific leaf area (SLA) and total leaf area (TLA) varied by taxa. TLA of *A. rubrum* and *Ulmus* 'Morton Glossy' trees were affected ($p \leq 0.05$) by irrigation rate (Table 2.4); however, the effect of irrigation treatment on SLA was not significant in any taxa. TLA was correlated (*A. freemanii*: $r=0.70$, $p \leq 0.0001$; *A. rubrum*: $r=0.64$, $p \leq 0.0001$; *Q. rubra*: $r=0.90$, $p \leq 0.0001$; *Ulmus* 'Morton': $r=0.58$, $p \leq 0.0001$; *Ulmus* 'Morton Glossy': $r=0.43$, $p \leq 0.0001$) with caliper growth in all of the taxa in which TLA was measured.

Irrigating in four cycles decreased ($p \leq 0.05$) foliar N concentration in *Q. rubra* and *Ulmus* 'Morton Glossy' trees compared to one cycle, while the high irrigation rate reduced foliar N concentrations in *Ulmus* 'Morton Glossy' trees ($p \leq 0.05$) and *A. rubrum* ($p \leq 0.01$) trees. The trend in mean foliar N content was *G. triacanthos* 'Harve' > *Q. rubra* > *A. freemanii* > *A. rubrum* > *G. triacanthos* 'Skyline' > *Ulmus* 'Morton' > *Ulmus* 'Morton Glossy'.

Absolute chlorophyll content (CC) was calculated from SPAD values according to Parry and Bugbee (2010). CC values were not correlated with foliar N in any taxa. CC leveled out by 2 June 2009 in *A. freemanii* trees. In 2008, CC content of *A. freemanii*, *G. triacanthos* 'Harve', and *G. triacanthos* 'Skyline' leaves declined between 15 September and 25 August. In the first year of the study, CC of *A. rubrum*, *Q. rubra* and *Ulmus* 'Morton', and *Ulmus* 'Morton Glossy' trees leveled out by sample 15 June, 5 August, and 28 August, respectively. In the second year of the study, CC of all species leveled out by 18 June, except for that of *Ulmus* 'Morton Glossy' which increased between 22 July and 11 August and that of *G. triacanthos* 'Skyline' which decreased between 1 July and 22 July.

There was no trend of foliar CC in response to irrigation treatment, and results varied by sampling date. Foliar CC of *G. triacanthos* 'Harve', *Ulmus* 'Morton', and *Q. rubra* trees was unaffected by irrigation treatment at all times. The low rate decreased ($p \leq 0.05$) CC of *A. freemanii*, *A. rubrum*, *Ulmus* 'Morton Glossy', and *G. triacanthos* 'Skyline' leaves on one date, regardless of the number of cycles in which irrigation was applied; however, the low rate increased CC in *A. freemanii* and *A. rubrum* leaves on one sampling date. Effect of cycle frequency, affecting CC of trees of only two taxa, was less apparent than irrigation rate. Increasing cycle frequency increased ($p \leq 0.05$) CC in only *A. rubrum* and *G. triacanthos* 'Skyline' trees on one date. There was an interaction between rate and frequency in *A. rubrum* trees during week of 2 June 2009; trees receiving the high rate in four cycles had higher ($p \leq 0.05$) CC than trees receiving the low rate in four cycles. The Fv/Fm values from chlorophyll fluorescence measurements varied by species, but there was no effect of irrigation treatment (data not shown).

Leachate

Effects of irrigation treatments on pour-through N concentration, pH, and EC varied by date of sampling. On 1 May 2009, the high irrigation rate reduced pour-through EC values and raised pH values compared to leachate from trees receiving the low rate. Leachate NO3-N and EC levels from trees receiving water in four cycles was less than that from leachate collected from trees receiving the same volume but delivered in one irrigation cycle only on one date (23 July 2009). Leachate collected from pour-throughs on this day from trees receiving the low rate in four cycles had EC values 50% less than samples from trees receiving the same volume but delivered in one cycle.

Discussion

This study was designed to test the effects of cyclic irrigation regimes on PIP-grown deciduous shade trees in Northern temperate climates. The overall goal was to develop scientifically based, water-conserving irrigation guidelines for growers in the upper Midwest. Specific objectives of this study were to 1) determine the effect of cyclic irrigation on the growth of deciduous shade trees, 2) explore the physiological responses which govern these growth responses, 3) examine the fate of nutrients in cyclic irrigation programs, and 4) compare various methods of calculating water use efficiency.

Growth responses

We did not observe the often cited increases in growth of container-grown plants in response to cyclic irrigation (Beeson and Haydu, 1995; Fain et al., 1999; Ismail et al., 2007; Keever and Cobb, 1985; Witmer, 2000). Most of these studies have been conducted in warmer climates with longer growing seasons where crops are subjected to harsher environmental conditions for prolonged periods of time. However, the 2009 growing season was unusually cool and wet; therefore it is possible that the stresses which cyclic irrigation would typically alleviate occurred less often or, when they did occur, were not as extreme as under typical conditions.

The taxa-specific differences in growth response to irrigation treatment indicate that variations in degrees of drought stress tolerance exist among taxa. Previous studies speculate that larger, rapidly growing taxa are more responsive to cyclic irrigation than smaller, slow-growing types (Beeson and Haydu, 1995; Witmer, 2000). However, growth of *G. triacanthos* 'Harve' and *Ulmus* 'Morton Glossy', the two fastest growing taxa (based on height growth) in our study, was unresponsive to irrigation cycle frequency.

We expected that responses in the second year of the study would be more reflective of treatments, compared to the first year, when trees were likely recovering from transplant stress. The enhanced growth response to irrigation rate in the second year of the study relative to the first year of the study could be a result of a larger tree root mass which is more responsive to soil moisture additions than less extensive root system (Beeson and Haydu, 1995; Witmer, 2000). Also in the second year of the study, trees were bigger, and as such had more transpirational area resulting in greater water demand.

Growth parameters varied in their sensitivity to irrigation regime. Stem radial growth was more responsive to irrigation treatment than height growth, as was expected. Height growth is completed relatively early in the season, while stem radial growth continues throughout the growing season. Therefore, stem radial growth is affected by season-long stresses of high temperature and VPD and reduced water availability.

Gas exchange

Past studies have documented higher mid-day g_S as a result of afternoon irrigation compared to once-daily morning applications (Beeson, 1992); however, we observed the opposite. A possible explanation for the reductions in mid-day g_S due to the cyclic irrigation could be due to the high VPD on the day of sampling which prevented the water applied at 1400 hr from penetrating the substrate profile. This was evident in diurnal substrate moisture plots.

Beeson (1992) attributed increases in plant growth to cumulative reductions in mid-day g_S due to cyclic irrigation. It is possible that the relatively cool and wet conditions of 2009 were not sufficient to limit mid-day g_S. Therefore, it is likely that the reason for the consistent growth across cycle frequencies was due to maintenance of g_S during the mid-day hours.

WUE

The sporadic response of mid-day WUE_i as a result of irrigation regime proves the parameter's limitations as an indicator of plant WUE. Because it is only a snapshot of plant function at one point in time, WUE_i does not accurately describe plant stress response. We found Δ to be a more sensitive indicator of plant stress than WUE_i, because it incorporates the accumulated isotopic differences over the entire course of tissue formation. Furthermore, because leaf synthesis is completed relatively early in the growing season, we expected the isotopic composition of wood tissue to be more sensitive to environmental stress during the growing season than that of foliar tissue. We observed similar or stronger responses in wood tissue compared to foliar tissue of the five taxa in which both of these carbon pools were sampled.

The lack of response of WUE_i to irrigation regime in the first year of the study could be due to the relatively small number of sampling events compared to the second year of the study. Container-grown *Q. rubra* trees in our study did not increase WUE_i under water-limited conditions, contrary to what has been reported in previous studies of mature forest trees (Turnbull et al., 2002).

Given similar atmospheric δ, differences in carbon isotopic composition of plant tissue is a function of stomatal aperture and δ carboxylation. Therefore, reductions in foliar Δ of *Q. rubra* and *Ulmus* 'Morton' trees indicate that the low irrigation rate was sufficient to decrease g_S or δ carboxylation during leaf synthesis and expansion. Reduced irrigation rates induced water stress in *Ulmus* trees during the growing season, evidenced by the decline of Δ values in *Ulmus* wood at reduced irrigation rates.

Carbon pools differ in their isotopic compositions (Bowling et al., 2008). In all taxa except *Acer* species, we observed the well-documented δ enrichment of wood tissue relative to bulk leaf matter, indicating postphotosynthetic carbon isotope fractionation (Damesin and Lelarge, 2003; Helle and Schleser, 2004; Brandes et al., 2006). Correlations of WUE_i and Δ have been recorded for a number of species (Roussel et al. 2009); however, we were able to correlate these parameters only in *Ulmus* trees, indicating the tight linkage between g_S and Δ in this species.

Leaf structure

Differences in late season patterns of CC values between years one and two could be due to variation in environmental conditions. In 2008, trees could have been responding to water and temperature stress by breaking down chlorophyll which was apparent in CC results. Mid-season declines in CC values of *G. triacanthos* trees could be associated with locust mites which were present on the trees at the beginning of the season in 2009. Studies have found that reduced leaching fractions due to cyclic irrigation regimes improves nutrient efficacy, thereby increasing foliar N concentrations compared to once daily applications (Allen et al., 2005; Karam et al., 1994; Tyler et al., 1996); however, we observed the opposite. Although the reason for this is still unclear, it is possible that, on days with high VPD, water applied during the 1400 hr cycle was not penetrating the substrate surface. Because controlled release fertilizers are dependent on water for nutrient release and movement of nutrients into roots is primarily a passive process, nutrients were not available for plant uptake. Similar declines in foliar nutrient (N, P, K, Ca, Mg) concentrations due to increasing plant moisture stress have been documented (Timmer and Armstrong, 1989; Turnbull et al., 2002).

The reductions in leachate NO3-N concentration due to cyclic irrigation compared to once daily applications concur with existing research (Fain et al., 1999; Fare et al., 1996). It is impossible to know whether cyclic irrigation regimes tested in our study resulted in less cumulative NO3-N leached from containers, because leaching fraction was not measured after each irrigation event.

Electrical conductivity levels exceeded the recommended limit of 0.5 dS/M for controlled release fertilizers. Leachate NO3-N concentration also exceeded the recommended 15 to 25 ppm (SNA, 2007) for all treatments on all sampling dates.

Conclusion

Cyclic irrigation did not affect growth of any of the species tested; however the reduced irrigation rate decreased growth in five of the seven taxa tested. It is likely that the beneficial effects of cyclic irrigation were underestimated, due to the mild summer of 2009. Growers should be more concerned with supplying a sufficient irrigation rate than the number of cycles in which it is delivered. Although the low rate reduces daily water applications and reduces input costs, it prolongs production time, ultimately reducing grower profitability. Modest reductions of less than 50% of the 2 cm rate, which is typical application, may not be detrimental to tree growth and should be explored in the future. WUE_i calculated from gas exchange measurements is limited in its accuracy as an indicator of plant water stress. Δ of plant tissue proved to be a sensitive indicator of plant water stress.

Table 2.1: Summary analysis of variance (ANOVA) for caliper and height growth of seven taxa of deciduous shade trees grown in #25 containers in a PIP production system under varying combinations of irrigation rate and cycle frequencies.

Source of variation	d.f.	Caliper	Height
Spp	6	43.95***	24.06***
Cycles	1	7.77	0.72
Spp*Cycles	6	1.27	1.16
Rate	1	64.31***	12.50**
Spp*Rate	6	2.11	1.4
Cycles*Rate	1	0.2	1.24
Spp*Cycles*Rate	6	0.58	0.87

Table 2.2: Summary repeated measures analysis of variance (ANOVA) for mid-day photosynthesis (A_{max}), leaf stomatal conductance (g_s), and WUE_i (A_{max}/g_s) of *A. freemanii*, *A. rubrum*, *Q. rubra*, *Ulmus* 'Morton', and *Ulmus* 'Morton Glossy' grown in #25 containers in a PIP production system under varying combinations of irrigation rate and cycle frequencies.

		F-values		
Source of Variation	d.f.	A_{max}	g_s	WUE_i
Between subjects				
Species (Spp)	4	15.04***	12.25**	4.57**
Cycles	1	2.64	0.53	0.17
Spp*Cycles	4	1.31	2.49	1.33
Rate	1	7.03*	10.69	0.47
Spp*Rate	4	0.65	0.62	0.12
Cycles*Rate	1	1.36	1.14	1.05
Spp*Cycles*Rate	4	0.29	0.25	0.60
Within subjects				
Date	4	65.68***	95.68***	20.10***
Date*Spp	16	3.19***	3.57***	3.09**
Date*Cycles	4	3.5**	8.09	2.91*
Date*Rate	4	2.33	1.72	0.20
Date*Spp*Rate	16	0.74	0.77	0.99
Date*Spp*Cycles	16	0.67	0.96	1.12
Date*Cycles*Rate	4	1.85	1.62	0.39
Date*Spp*Cycles*Rate	16	0.65	0.54	0.43

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

Table 2.3: Mean mid-day net photosynthesis (A_{max}; $\mu mol \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s; mol $H_2O \cdot m^{-2} \cdot s^{-1}$), and intrinsic water use efficiency (WUE_i; A_{max}/g_s) (±SE) of five taxa of deciduous shade trees grown in #25 containers in a PIP facility under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four). Means with different letters are statistically different at $p \leq 0.05$. Data averaged across all sampling dates and irrigation treatments.

Taxa	g_s	A_{max}	WUE_i
A. freemanii	0.065±0.004a	6.780±0.38a	122.07±6.76a
A. rubrum	0.096±0.007ab	8.14±0.43ab	96.71±3.05bc
Q. rubra	0.069±0.004a	6.70±0.35bc	112.85±6.44ab
Ulmus 'Morton'	0.113±0.007bc	9.40±0.49cd	89.60±2.79c
Ulmus 'Morton Glossy'	0.142±0.009c	10.90±0.50d	87.38±2.15c

Table 2.4: Specific leaf area (SLA), total leaf area (TLA), foliar N concentration, and chlorophyll content (CC) of seven taxa of deciduous shade trees grown in #25 containers under two irrigation rates (low, 1 cm or high, 2 cm) on 27 July 2009. Within columns, means with different letters are different at p≤0.05. Data averaged across all cycle frequencies.

Taxa	Rate	SLA (g/cm^2)	TLA (m^2)	Foliar N (%)	CC (mol/m^2)
A. freemanii	Low	133.19	4.04	3.45	26.78
	High	128.23	5.07	3.33	24.67
	Overall	130.88A	4.52B	3.39BC	25.73C
A. rubrum	Low	121.91	6.03a	3.45b	42.52
	High	125.43	9.16b	3.18a	43.25
	Overall	123.67A	7.59A	3.32BC	42.88B
G. triacanthos 'Harve'	Low	----	----	3.97	44.29
	High	----	----	3.83	46.71
	Overall	----	----	3.90A	45.42B
Q. rubra	Low	129.29	5.65	3.55	26.35
	High	128.64	7.46	3.51	26.22
	Overall	128.99A	6.50A	3.53BA	26.29C
G. triacanthos 'Skyline'	Low	----	----	3.26	24.07
	High	----	----	3.19	22.95
	Overall	----	----	3.23BC	23.51C
Ulmus 'Morton'	Low	117.04	6.15	3.19	56.06
	High	113.64	8.19	3.05	52.20
	Overall	115.34A	7.17A	3.12DC	54.13A
Ulmus 'Morton Glossy'	Low	96.37	5.58a	3.02b	46.31
	High	102.37	7.72b	2.84a	47.61
	Overall	99.37B	6.65A	2.93D	46.96BA

Figure 2.1: Weekly rainfall and reference evapotranspiration (ET_0) from Michigan Automated Weather Network at Michigan State University (MSU) Hancock Turfgrass Research Center (2008) and MSU Horticulture Teaching and Research Center, East Lansing, MI. Rainfall deficit = ET_0 – rainfall.

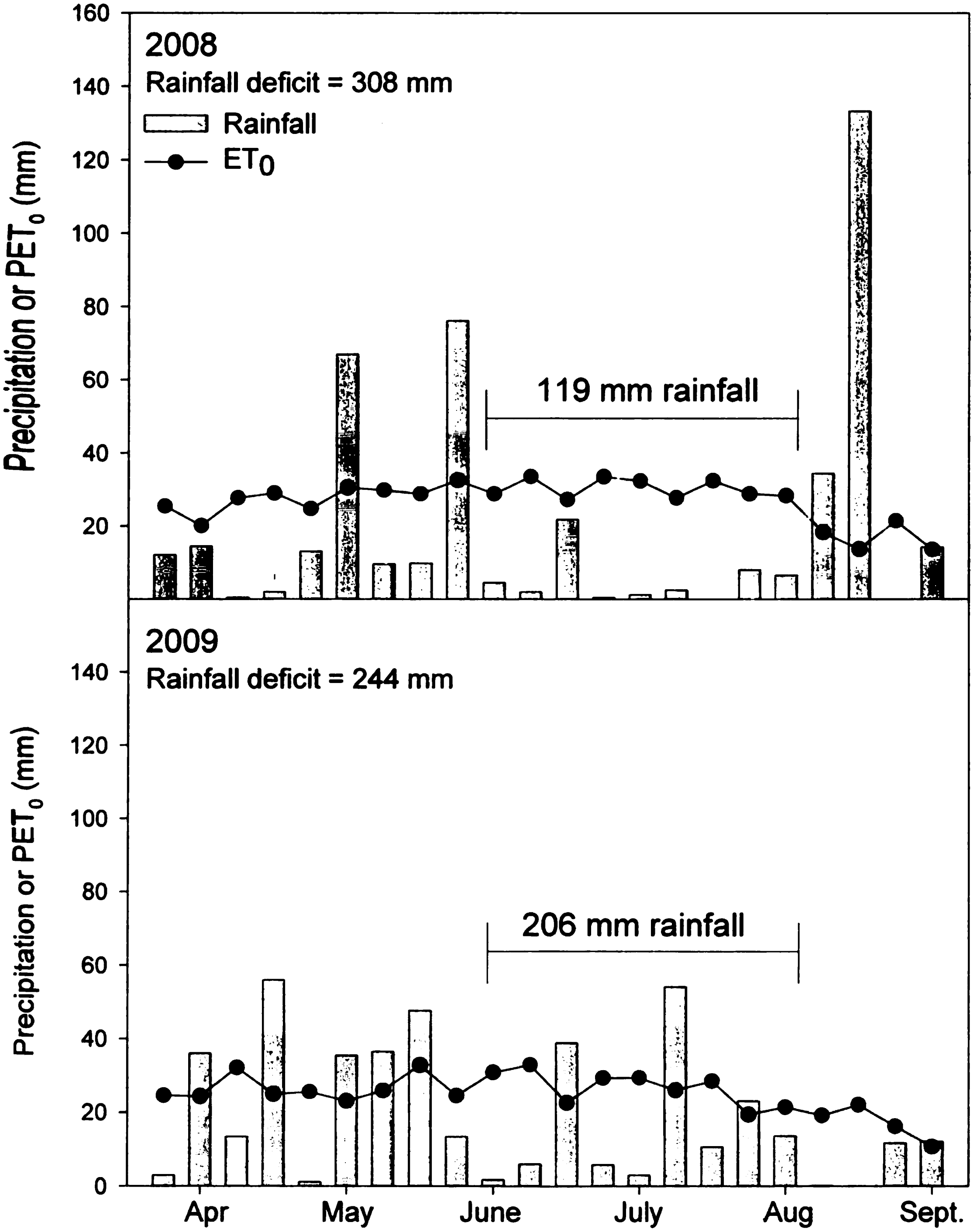


Figure 2.2: Container capacity and seasonal course of substrate volumetric moisture content (VWC; %) of a subsample of 16 deciduous shade trees grown in #25 containers in a PIP facility under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four).

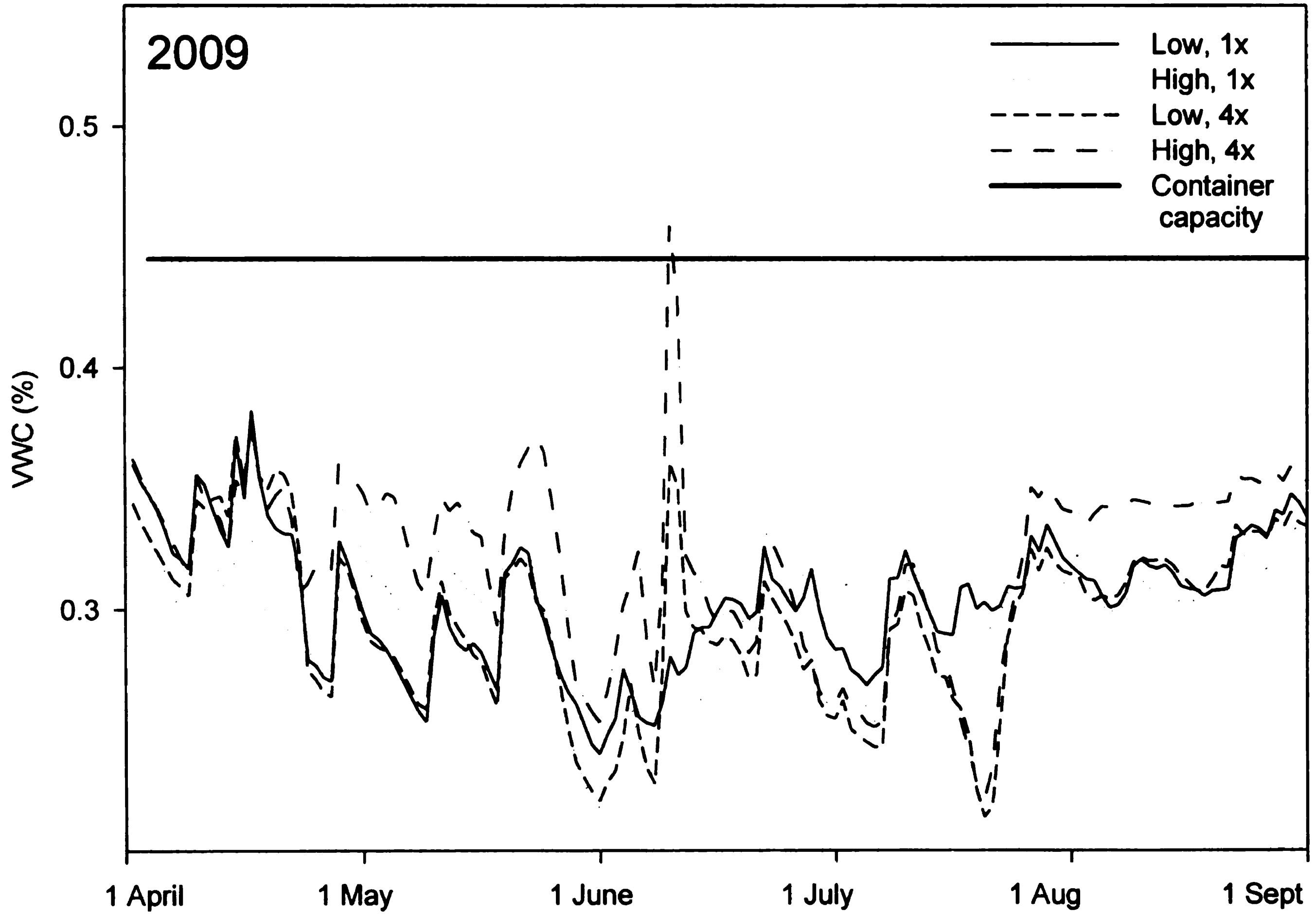


Figure 2.3: Stem radial growth (±SE) over the two-year production cycle of seven taxa of deciduous shade trees grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four). Effect of cycles was not significant. Data averaged across cycles (n=8; * $p \leq 0.05$; ** $p \leq 0.01$).

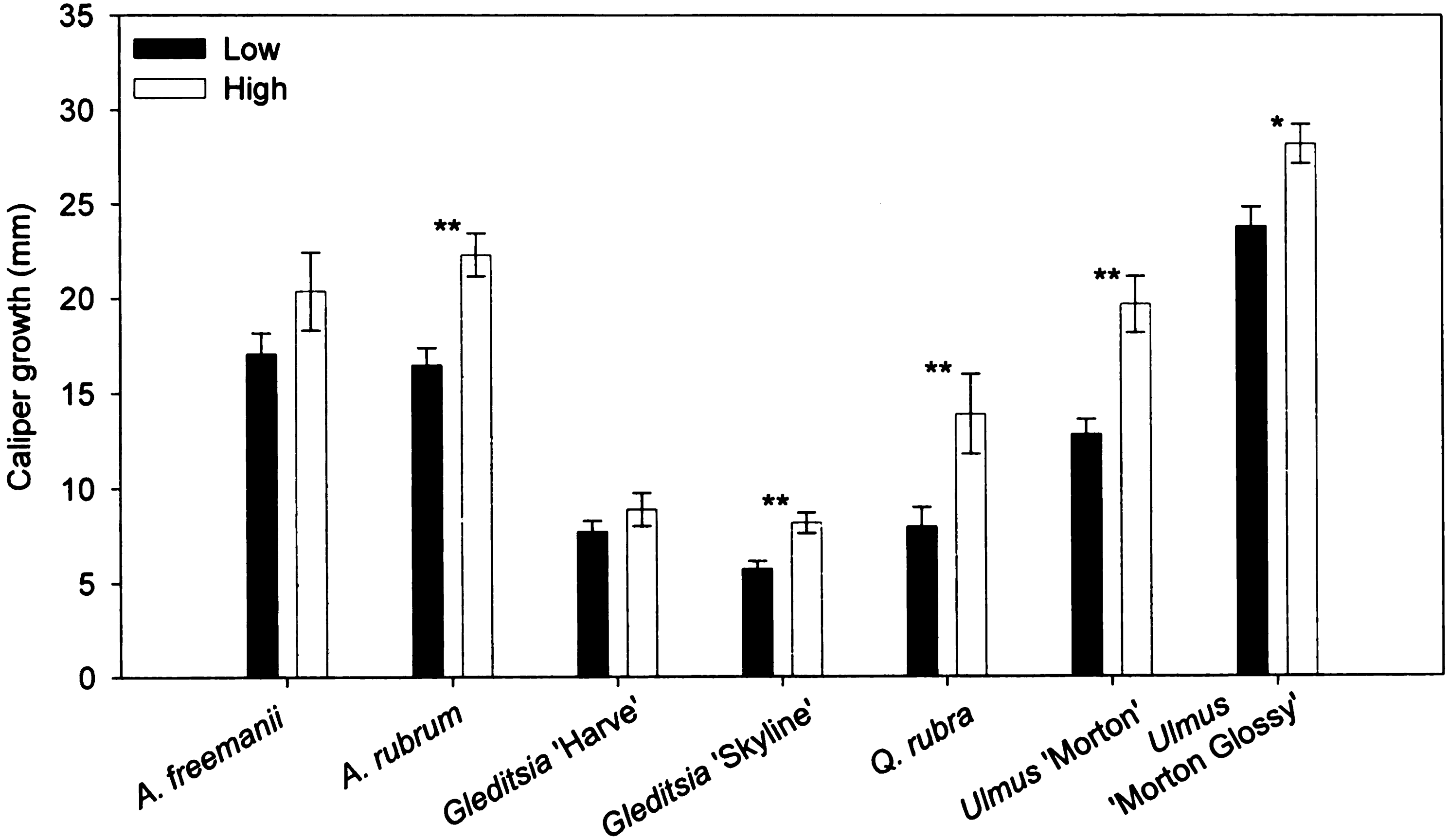


Figure 2.4: 2009 mid-day leaf stomatal conductance (g_S; mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) of five taxa of deciduous shade trees grown in #25 containers in a PIP production system under two irrigation rates (low, 1 cm or high, 2 cm). Data averaged across both irrigation rates (n=40).

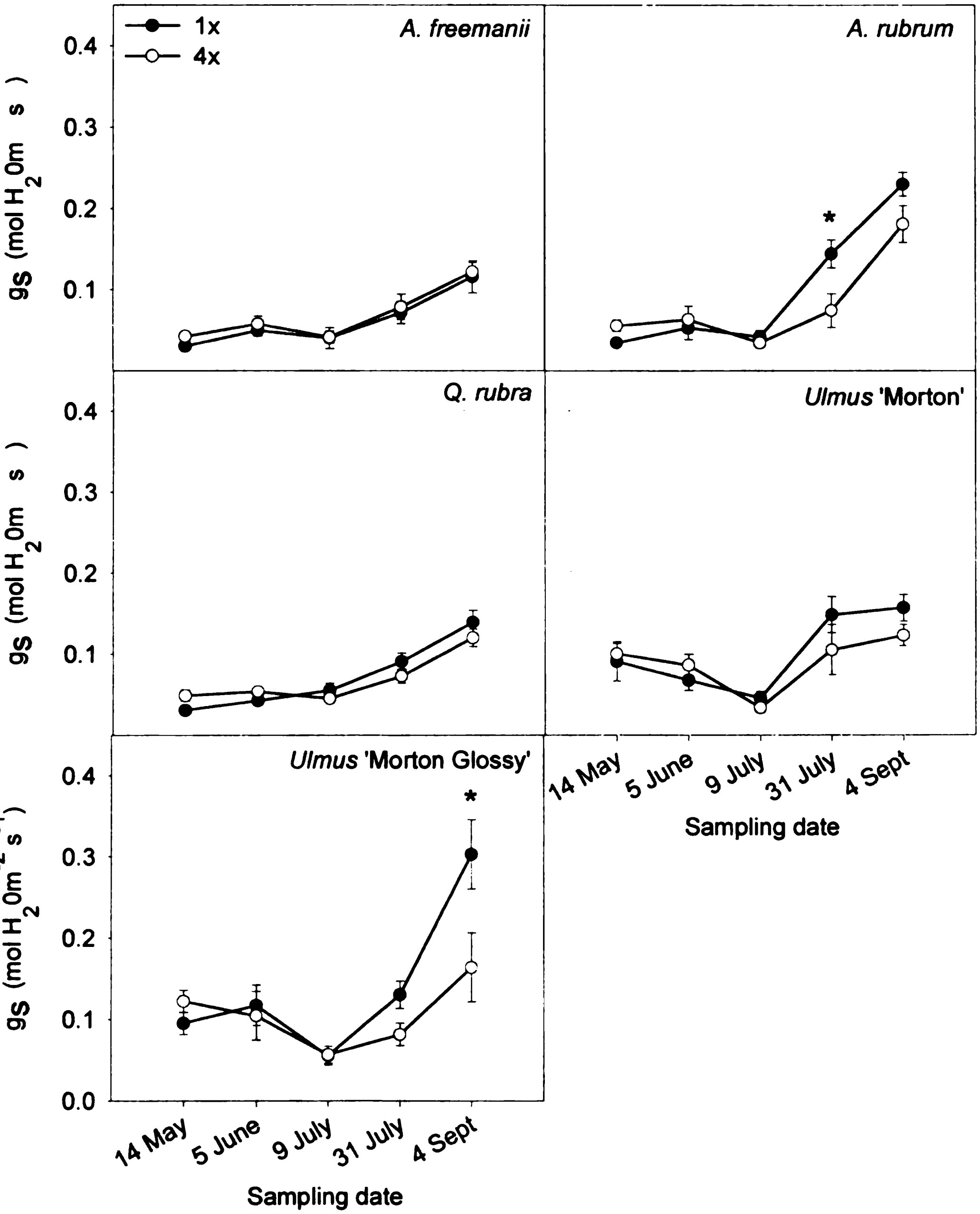


Figure 2.5: 2009 mid-day net photosynthetic rate (A_{max}; $\mu mol \cdot m^{-2} \cdot s^{-1}$), leaf stomatal conductance (g_s; mol $H_2O \cdot m^{-2} \cdot s^{-1}$), and instantaneous water use efficiency (WUE_i; A_{max}/g_s) of five taxa of deciduous shade trees grown in #25 containers in a PIP production system under two irrigation rates (low, 1 cm or high, 2 cm). Effect of cycle frequency and irrigation rate was not significant. Data averaged across both cycle frequencies (n=40).

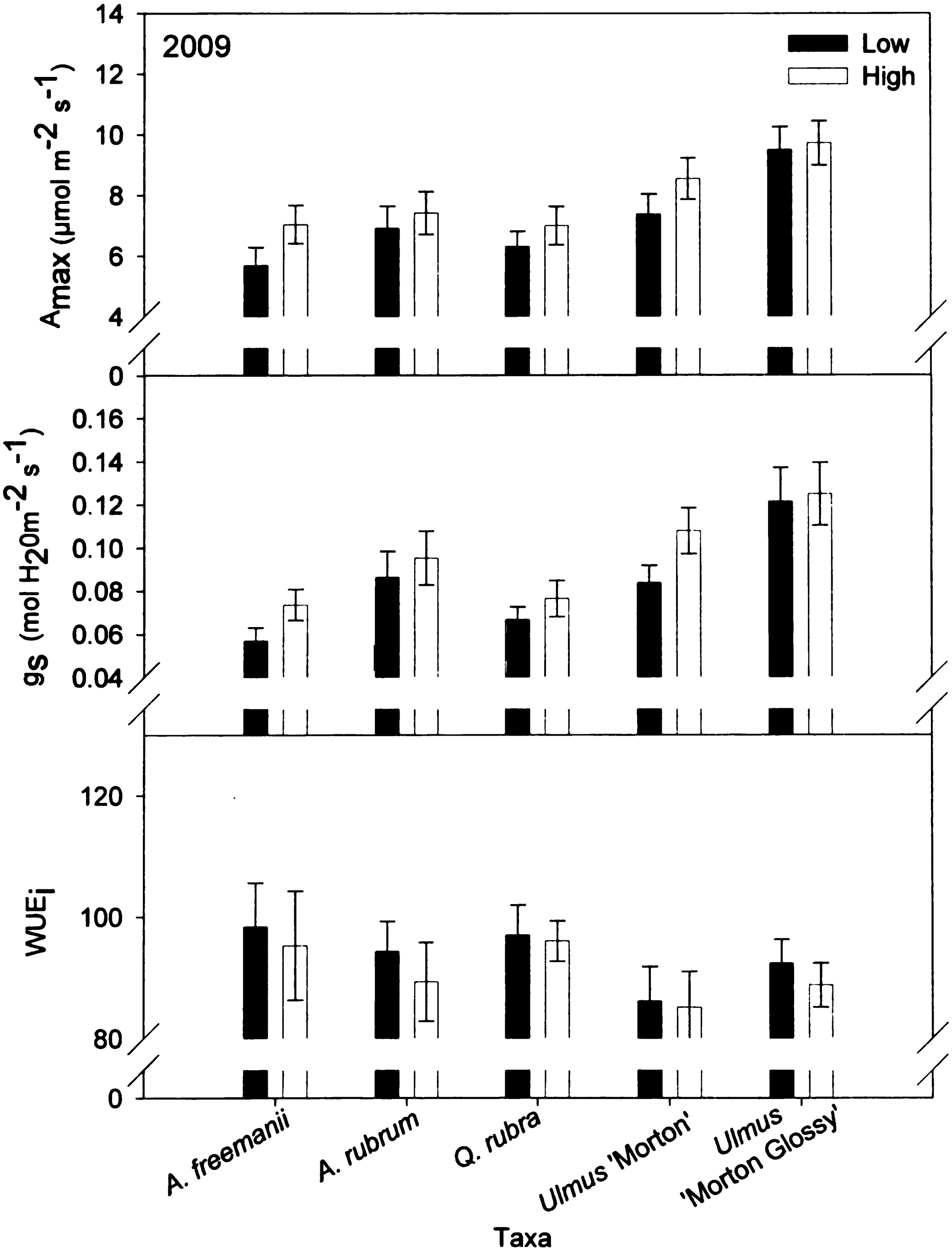


Figure 2.6: Diurnal courses of stomatal conductance (g_S; mol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$) of *Acer freemanii and Acer rubrum* trees grown in #25 containers in a PIP production system in 2008 and 2009 under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and cycle frequency (one or four). Data averaged across irrigation rate and cycle frequency when effect of treatment was not significant (n=16). Means with different letters are significantly different at $p \leq 0.05$.

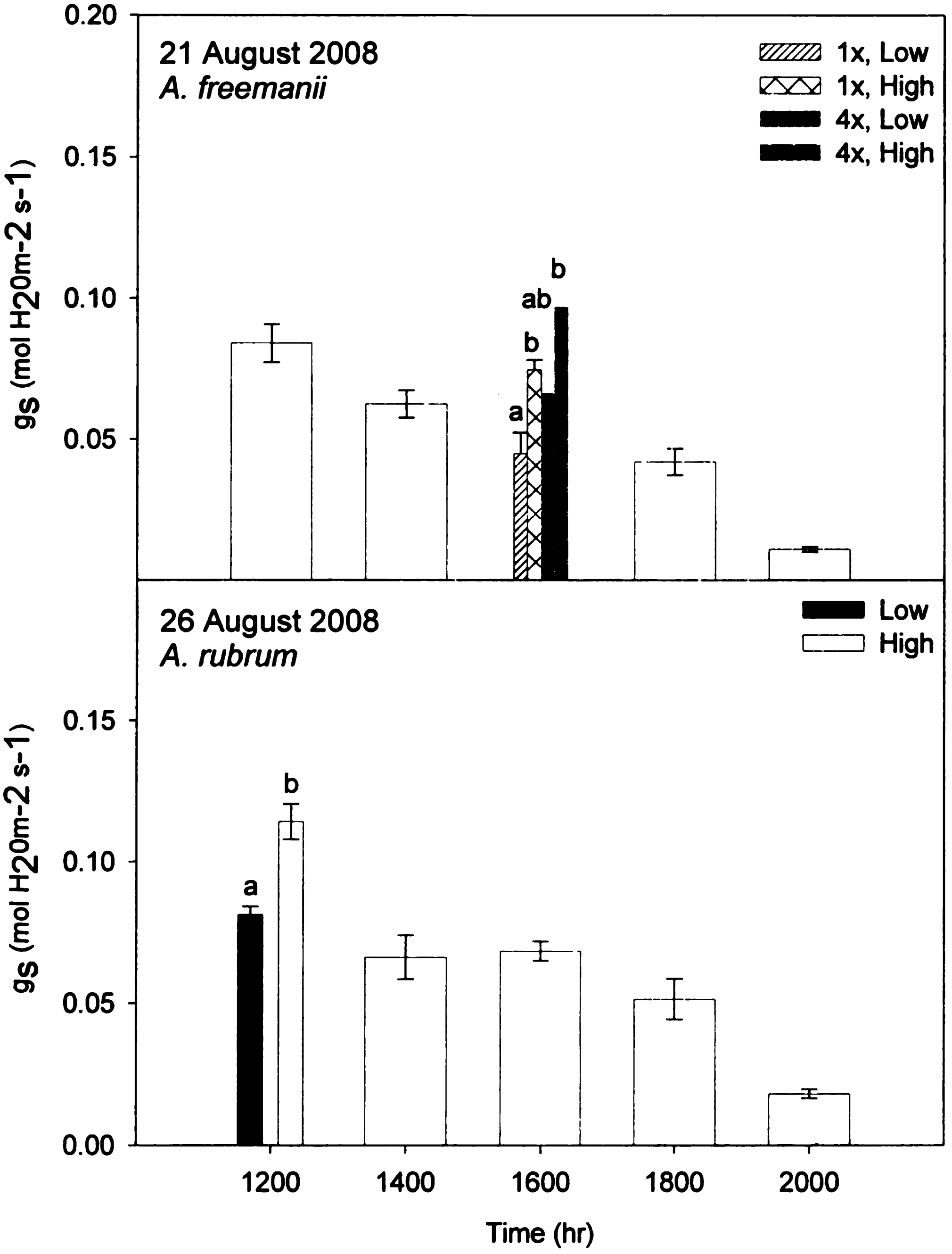


Figure 2.7: Diurnal course of leaf water potential (MPa) of *Quercus rubra* trees grown in #25 containers in a PIP production system in 2008 and 2009 under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and cycle frequency (one or four). Effect of cycles was not significant at any time. Data averaged across irrigation rate and cycle frequency when effect of treatment was not significant (n=16). Times when irrigation rate was significant, data averaged across both irrigation cycle frequencies (n=8; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.0001$).

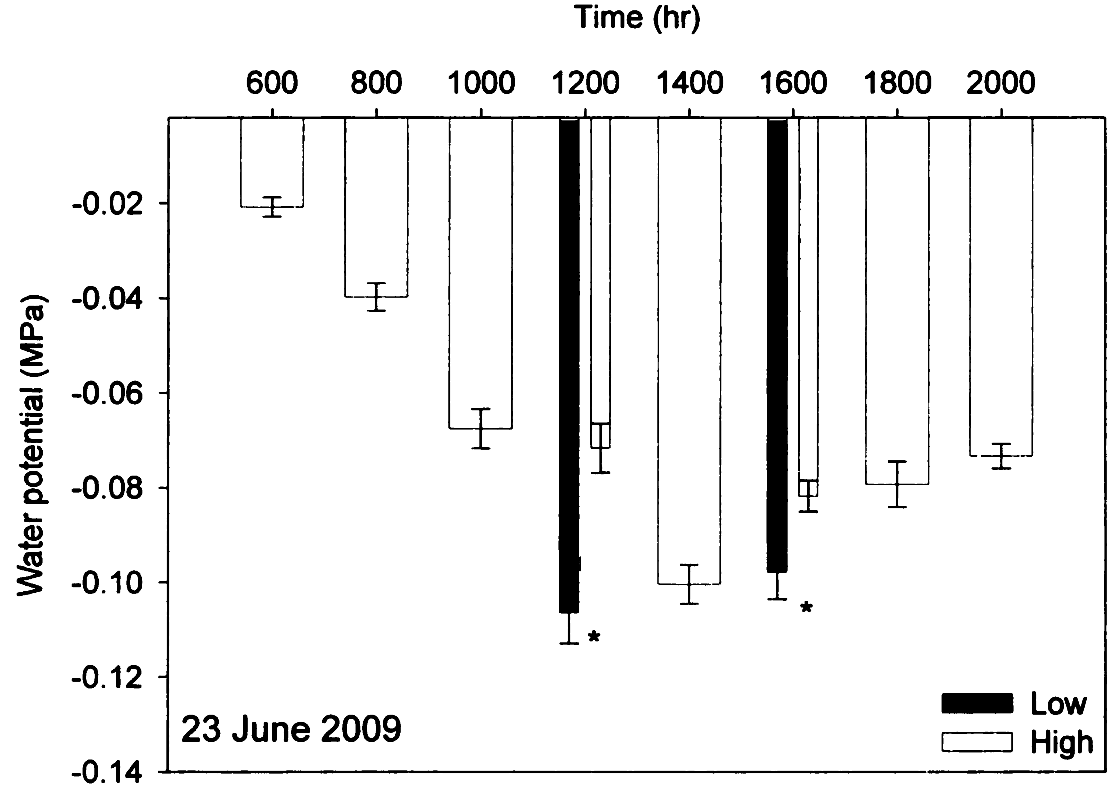


Figure 2.8: Diurnal course of leaf water potential (MPa) of *Acer freemanii* trees grown in #25 containers in a PIP production system in 2008 and 2009 under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and cycle frequency (one or four). Effect of cycles was not significant. Data averaged across irrigation rate and cycle frequency when effect of treatment was not significant (n=16). Times when irrigation rate was significant, data averaged across both irrigation cycle frequencies (n=8; * p≤0.05; ** p≤0.01).

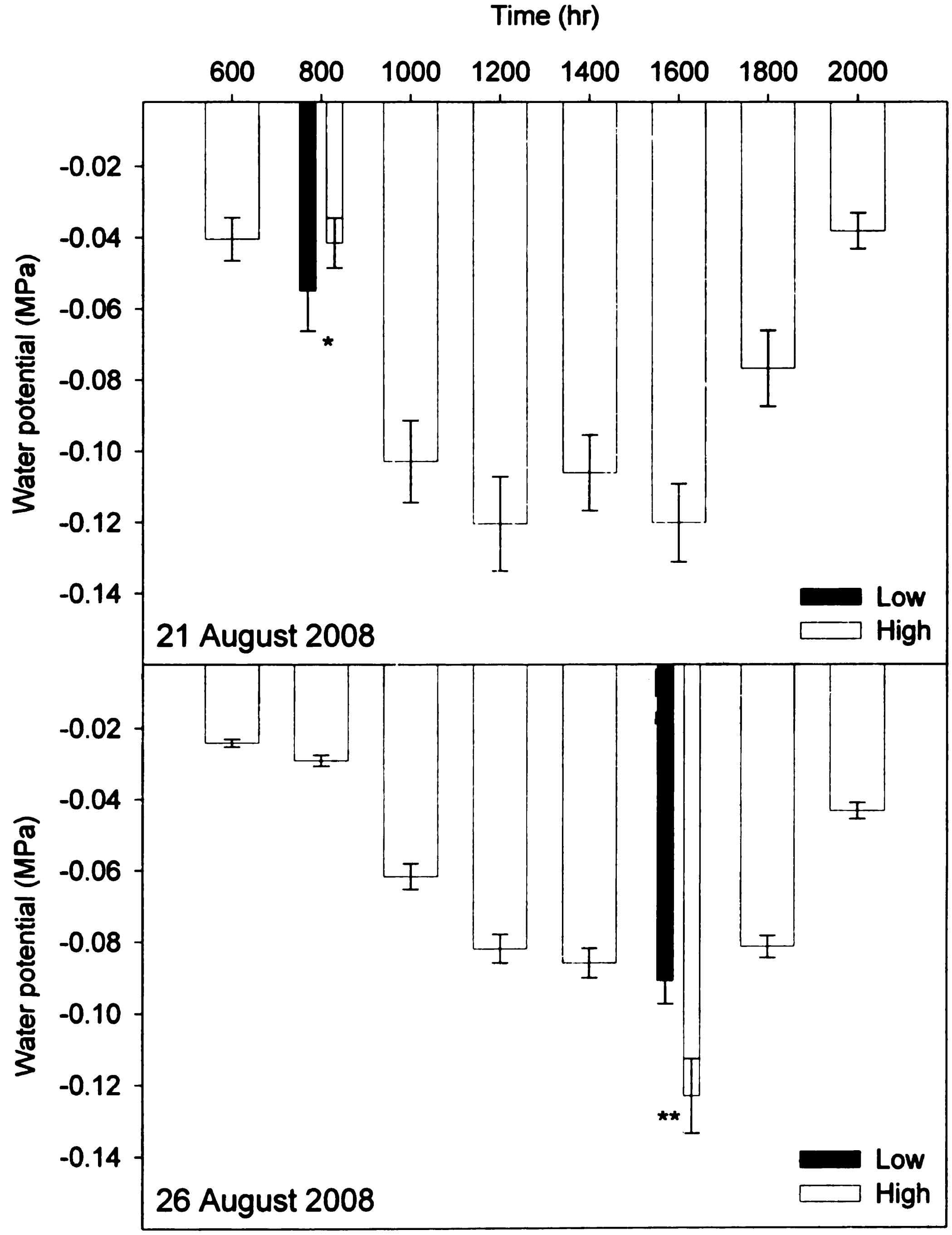


Figure 2.9: Δ values (‰) (±SE) of 2009 foliar tissue of five deciduous shade tree species grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) (n=8; * $p \leq 0.05$; ** $p \leq 0.01$). Effect of cycles was not significant in any taxa except *A. rubrum*, in those taxa data is averaged across cycle frequencies. In graph of *A. rubrum*, means with different letters are statistically different at $p \leq 0.05$.

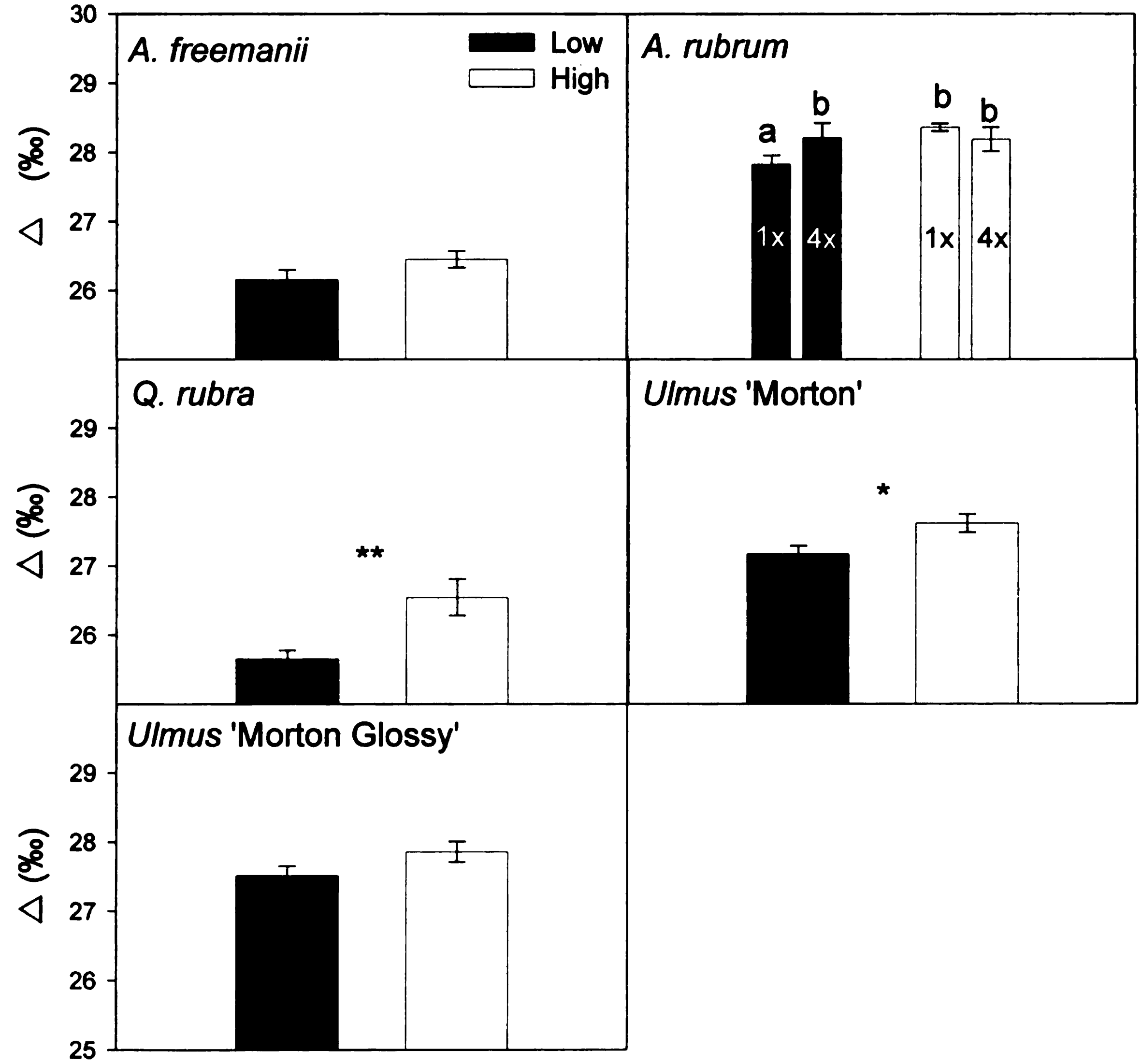


Figure 2.10: Δ values (‰) (±SE) of 2008 and 2009 wood of ***Acer rubrum*** trees grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) (n=16; ** $p \leq 0.01$). Effect of irrigation rate was not significant; data averaged across rates.

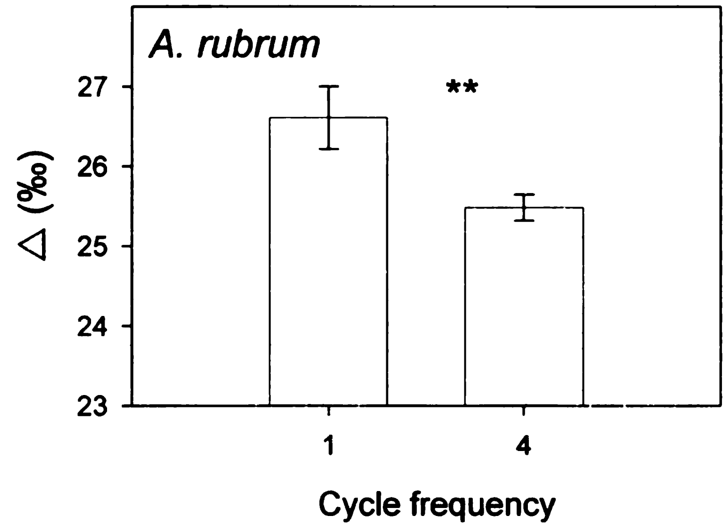
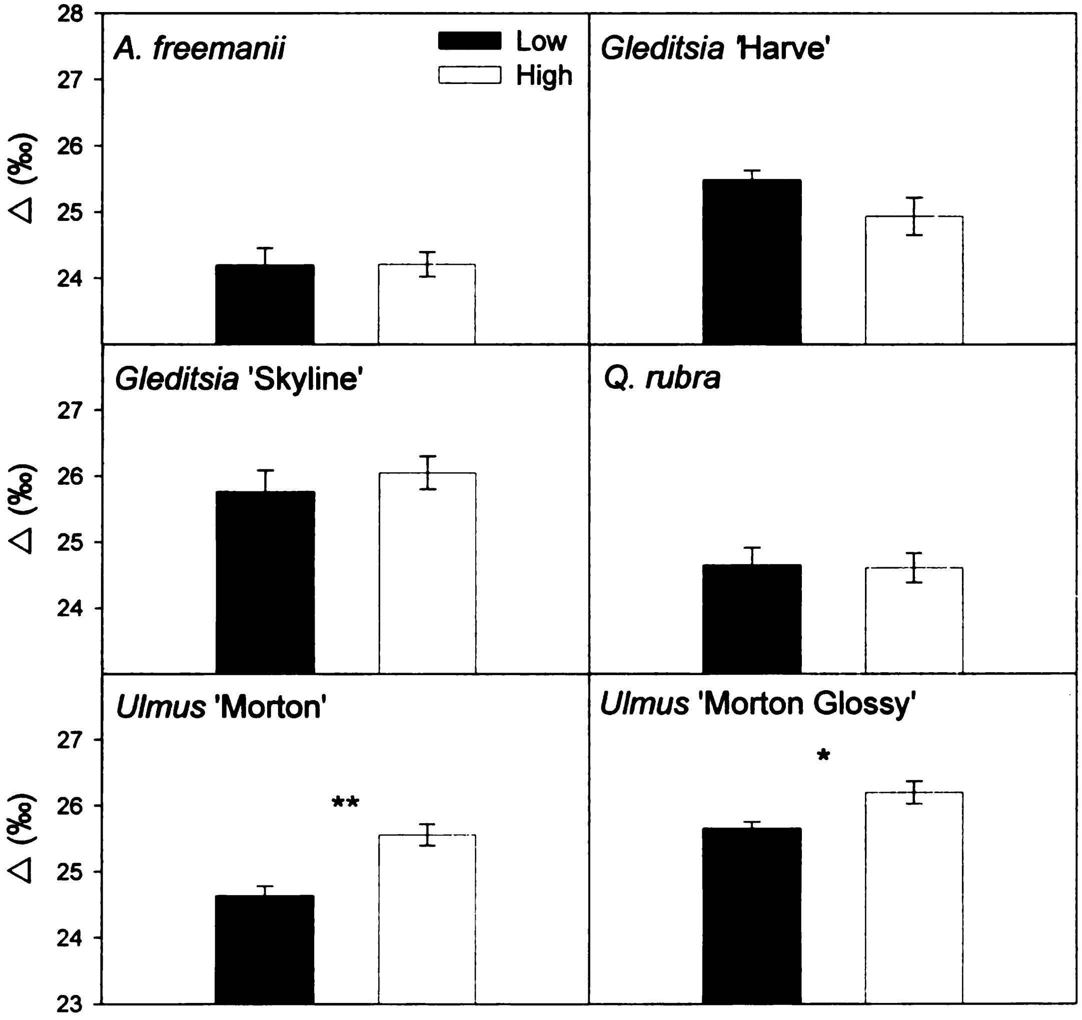


Figure 2.11: Δ values (‰) (±SE) of 2008 and 2009 wood of six deciduous shade tree species grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) (n=16; * p≤0.05; ** p≤0.01). Effect of cycle frequency was not significant.



LITERATURE CITED

Allen, C. B., R. E. Will, and M.A. Jacobson. 2005. Production efficiency and radiation use efficiency of four tree species receiving irrigation and fertilization. Forest Science 51(6): 556-569.

Anyia, A.O. and H. Herzog. 2004. Water use efficiency, leaf area, and leaf gas exchange of cowpeas under mid-season drought. Europ. J. Agronomy 20:327-339.

Bacelar, E.A., Moutinho-Pereira, J.M., Goncalves, B.C., Ferreira, H.F., and C.M. Correia. 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environmental and Experimental Botany 60:183–192.

Beeson, Jr., R.C. 1992. Restricting overhead irrigation to dawn limits growth in container-grown woody ornamentals. Hortscience 27(9): 996-999.

Beeson, Jr., R. C. and J. Haydu. 1995. Cyclic microirrigation in container-grown landscape plants improves plant growth and water conservation. Journal of Environmental Horticulture 13(1): 6-11.

Beeson, Jr., R. C., M. A. Arnold, T.E. Bilderback, B. Bolusky, S. Chandler, H.M. Gramling, J.D. Lea-Cox, J. R. Harris P.J. Klinger, H.M. Mathers, J.M. Ruter, and T.H. Yeager. 2004. Strategic vision of container nursery irrigation in the next ten years. Journal of Environmental Horticulture 22(2): 113-115.

Bilderback, T.E. 2001a. Environmentally compatible container plant production practices. Proc. Int. Symp. on Growing Media & Hydroponics. Acta Hort. 548. International Society of Horticultural Science.

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

Bowling, D.R. D.E. Pataki, and J.T. Randerson. 2008. Carbon isotopes in terrestrial ecosystem pools and CO2 fluxes. New Phytologist 178:24-40.

Brandes, E., N. Kodama, K. Whittaker, C. Weston, H. Rennenberg, C. Keitel, M.A. Adams, and A. Gessler. 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. Global Change Biology 12:1922-1939.

Bsoul, E., R. St Hilaire, and D.M. VanLeeuwen. 2007. Bigtooth maples from selected provenances effectively endure deficit irrigation. Hortscience 42(5): 1167-1173.

Cernusak, L. A., K. Winter, and B.L. Turner. 2009. Physiological and isotopic (delta13C and delta18O) responses of three tropical tree species to water and nutrient availability. Plant, Cell and Environment 32(10): 1441-1455.

Cregg, B. and J. Zhang. 2000. Carbon isotope discrimination as a tool to screen for improved drought tolerance. Proceedings of the 11th Metropolitan Tree Improvement Alliance (METRIA) Conference, Gresham, OR.

Damesin, C. and C. Lelarge. 2003. Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. Plant, Cell and Environment 26(2):207-219.

Fain, G. B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1999. Cyclic irrigation improves irrigation application efficiency and growth of sawtooth oak. Journal of Arboriculture 25(4): 200-203.

Fare, D. C., C.H. Gilliam, G.J. Keever, and R.B. Reed. 1996. Cyclic irrigation and media affect container leachate and ageratum growth. Journal of Environmental Horticulture 14(1): 17-21.

Gouveia, A.C. and H. Freitas. 2009. Modulation of leaf attributes and water use efficiency in Quercus suber along a rainfall gradient. Trees 23:267–275.

Groves, K. M., S. L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizers: I. Effect on plant growth and mineral nutrient content in containerized plant production. Journal of Environmental Horticulture 16(3): 176-181.

Hand, M. J., E. Young, and A.C. Vanconcelos. 1982. Leaf water potential, stomatal resistance, and photosynthetic response to water stress in peach seedlings. Plant Physiology 69: 1051-1054.

Helle, G. and G.H. Schleser, 2004. Beyond CO2-fixation by Rubisco – an interpretation of 13C/12C variations in tree rings from novel intra-seasonal studies on broad-leaf trees. Plant, Cell, and Environment 27, 367–380.

Huffman, S.A. and K.A. Barbarick. 1981. Communications in soil science and plant analysis. 12(1):79-89.

Ismail, S.M., K. Ozawa, and N.A. Khondaker. 2007. Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. First Power). Agricultural Water Management 95(2008): 116-122.

Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam, and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies 331(1): 42-47.

Karam, F., R. Masaad, R. Bachour, C.Rhayem, and Y. Rouphael. 2009. Water and radiation use efficiencies in drip-irrigated pepper (*Capsicum annuum* L.): response to full and deficit irrigation regimes. European Journal of Horticultural Science 74(2): 79-85.

Keever, G. J. and G. S. Cobb. 1985. Irrigation scheduling effects on container media and canopy temperatures and growth of 'Hershey Red' azalea. Hortscience 20(5): 921-923.

Keitel,C., A. Matzarakis, H. Rennenberg, and A. Gessler. 2006. Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. Plant, Cell and Environment 29:1492–1507.

Martin, C.A., H.G. Ponder, and C.H. Gilliam. 1989. Effects of irrigation rate and media on growth of *Acer rubrum* L. in containers. Journal of Environmental Horticulture 7(1): 38-40.

Medrano, H., J.M. Escalona, J. Bota, J. Gulias, and J. Flexas. 2002. Regulation of photosynthesis of C-3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Annals of Botany 89: 895-905.

Merchant, A., A.D. Peuke, C. Keitel, C. Macfarlane, C.R. Warren, and M.A. Adams. 2010. Phloem sap and leaf δ^{13}C, carbohydrates, and amino acid concentrations in Eucalyptus globulus change systematically according to flooding and water deficit treatment. Journal of Experimental Botany 61(6): 1785–1793.

Ningbo, C., T. Dub, S. Kang, F.Li, X. Hua, M. Wang, and Z. Li. 2009. Relationship between stable carbon isotope discrimination and water use efficiency under regulated deficit irrigation of pear-jujube tree. Agricultural Water Management 96: 1615–1622.

Parry, C. and B. Bugbee. 2010. Leaf chlorophyll concentration: Linking relative and absolute measurements. USDA NCERA-101 Committee on Controlled Environment Technology & Use, Annual Mtg. Madison, WI.

Roberts, B.R. and V.M. Schnipke. 1987. The water requirements of five container-grown *Acer* species. Hortscience 22(5):1161-1161.

Roussel, M., E. Dreyer, P. Montpied, G. Le-Provost, J.M. Guehl, and O. Brendel. 2009. The diversity of ^{13}C isotope discrimination in a *Quercus robur* full-sib family is associated with differences in intrinsic water use efficiency, transpiration efficiency, and stomatal conductance. Journal of Experimental Botany 60(8):2419-2431.

Ruter, J.M. 1998. Pot-In-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. Journal of Environmental Horticulture 16(3): 159-162.

Seibt, U., A. Rajabi, H. Griffiths, and J.A. Berry. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155:441–454.

SNA. 2007. Best Management Practices Guide 2.0. Southern Nursery Association.

Taiz, L. and E. Zeiger. Plant Physiology, 4th edn. Sunderland, MA: Sinauer Associates Inc., 2006.

Timmer, V.R. and G. Armstrong. 1989. Growth and nutrition of containerized *Pinus resinosa* seedlings at varying moisture regimes. New Forests 3: 171-180.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F Schuster, K.J. Brown, V.C. Engel, and K.L. Griffin. 2002. Photosynthetic characteristics in canopies of *Quercus rubra, Quercus prinus* and *Acer rubrum* differ in response to soil water availability. Oecologia 130:515–524.

Tyler, H. H., S. L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. Journal of Environmental Horticulture 14(4): 199-204.

Warren, S.L. and T.E. Bilderback. 2002. Timing of low pressure irrigation affects plant growth and water utilization efficiency. Journal of Environmental Horticulture 20(3): 184-188.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009a. Water conservation, growth, and water use efficiency of container-grown woody ornamentals irrigated based on daily water use. Hortscience 44(5): 1308-1318.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009b. Container-grown ornamental plant growth and water runoff nutrient content and volume under four irrigation treatments. Hortscience 44(6): 1573-1580.

Witmer, R. 2000. Water use of landscape trees during pot-in-pot production and during establishment in the landscape. Horticulture. Blacksburg, Virginia Polytechnic Institute and State University. Doctor of Philosophy: 195.

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

Yoo, C.Y., H.E. Pence, P.M. Hasegawa, and M.V. Mickelbart. 2009. Regulation of transpiration to improve crop water use. Critical Reviews in Plant Sciences 28: (6)410-431.

CHAPTER THREE

GROWTH AND PHYSIOLOGY OF CONTAINER-GROWN CONIFERS UNDER CYCLIC IRRIGATION

Abstract

Cyclic irrigation, applying a plant's daily water allowance in multiple allotments, is effective in increasing growth in container-grown plants. The objective of this study was to quantify the effects of cyclic irrigation regimes on the growth and physiology of common container-grown conifer species in Pot-in-Pot (PIP) production in the upper Midwest. Four species of conifers (*Picea glauca* var. *densata*, *Picea pungens*, *Abies fraseri*, and *Pinus strobus*), grown in #3 (10.2-L) and #7 (24.5 L) containers, were randomly assigned to irrigation regimes with varying combinations of irrigation rates (low, medium, or high) and daily cycle frequencies (one or four). Low, medium, and high irrigation rates of trees grown in the #3 containers corresponded to 1 cm, 2 cm, and 3 cm depths, respectively. Low and high rates of trees grown in the #7 containers corresponded to 1 cm and 2 cm depths, respectively. Height growth of only *P. strobus* trees increased under cyclic irrigation regimes, and irrigation rates did not affect height growth. Physiological parameters were more responsive to irrigation rate than cycle frequency. Mid-day gas exchange and instantaneous water use efficiency (WUE_i) were unaffected by irrigation treatment. Transpiration efficiency and Δ in foliar and wood tissue increased at reduced irrigation rates. Increasing cycle frequency increased Δ in foliar and wood tissue. Irrigation rates of 2 cm leached more NO_3-N from containers and reduced foliar N content of *P. strobus* and *P. pungens* trees grown in #7 containers compared to the 1 cm rate. Trees of equal size can be produced with 50% less water than traditional methods, increasing profitability for growers by reducing input costs.

wat

dis

(Ba

exc

gro

and

the

(Bi

wi

fut

wa

hig

yie

al.

ca

mo

19

vol

Ma

Introduction

Increased water costs, decreased water availability due to increased competition for water resources with urban areas, and anticipation of stricter water withdrawal and discharge regulations have caused nursery producers to rethink water use practices (Beeson et al., 2004). Growers commonly overwater, applying a fixed rate of irrigation exceeding plant water use, which may result in excessive water withdrawals. Container growing also required the addition of fertilizer for optimum crop growth. The resulting, and often highly concentrated, container solution is highly susceptible to leaching, due to the low cation exchange capacity of commonly used pine-bark based substrates (Bilderback, 2001a). The development of irrigation programs which conserve water without sacrificing tree growth and quality will allow the nursery industry to adapt to future water use regulations, increased input costs, and decreased water availability.

One option to conserve water is cyclic irrigation, where fractions of a plant's daily water allowance is applied several times daily. Plant responses to cyclic irrigation include higher growth index, root growth, trunk diameter, shoot dry weight, height, and crop yield than plants receiving irrigation in only one cycle (Beeson and Haydu, 1995; Fain et al., 1999; Ismail et al., 2007; Keever and Cobb, 1985; Witmer, 2000). In some cases, caliper growth was 25% greater using cyclic irrigation compared to the traditional method of applying a single cycle in the morning (Beeson and Haydu, 1995; Fain et al., 1999; Witmer, 2000).

Equal or superior growth rates of woody ornamental crops at reduced irrigation volumes have also been documented (Beeson and Haydu, 1995; Groves et al., 1998; Martin et al., 1989; Roberts and Schnipke, 1987; Warsaw et al., 2009a; Warsaw et al., 2009

volu

spec

irrig

pro

Zei

(Ha

ass

or r

and

(W

pro

in

20

co

tra

the

(or

2009b). Beeson and Haydu (1995) summarized that under cyclic irrigation the water volume required per area can be reduced by at least 25% and 50% for xeric or mesic species, respectively, with no effect on growth (compared to standard overhead irrigation). However, there may be a trade-off between water conservation and plant productivity if irrigation volume does not meet plant demand (Groves et al., 1998).

Up to 97% of water taken up by a plant is transpired through stomata (Taiz and Zeiger, 2006), and the initial response to limited water availability is stomatal closure (Hand et al., 1982; Medrano et al., 2002), which inhibits CO_2 uptake and reduces assimilation. It has been hypothesized that cyclic irrigation increases growth by removing or reducing mid-day water limitations, thereby delaying or preventing stomatal closure and increasing the cumulative time of photosynthesis (Beeson, 1992; Witmer, 2000).

The relationship between transpiration and assimilation is the water use efficiency (WUE) and can be viewed as a measure of how efficiently a plant is using water to produce biomass (Anyia and Herzog, 2004). Several studies have demonstrated increases in WUE when trees are exposed to water limiting irrigation regimes (Anyia and Herzog, 2004; Ningbo et al., 2009; Warren and Bilderback, 2002). There are three methods commonly used to calculate WUE: instantaneous WUE, integrated WUE, and transpiration efficiency.

Instantaneous WUE (WUE_i) measures WUE at the leaf level, and is the ratio of the rate of CO_2 assimilation (measured at the leaf level) ratio to rate of water transpired (or the rate of CO_2 uptake). It is calculated using gas exchange data as:

Insta

phot

chan

200

with

incr

env

^{13}(

pro

disc

ligh

dec

stre

to r

in th

resp

A/E or A/g_s

Instantaneous WUE excludes carbon losses associated with respiration and non-photosynthetic functions and is referred to as intrinsic WUE, because the response of E to changes in g_s and vapor pressure deficit (VPD) is inherent to a species (Bauerle et al., 2006; Bsoul et al., 2007; Cernusak et al., 2009). Reductions in transpirational losses without concomitant reductions in A can increase WUE_i and lead to substantial long-term increases in WUE (Yoo et al., 2009).

Stable carbon isotope analysis has become an important method of assessing environmental stress. Carbon isotope discrimination (**Δ**), using the ratio of intercellular ^{13}C to ^{12}C, is used to calculate integrated water use efficiency, so-called because it provides a cumulative measure of plant status over the time tissues were formed. Plants discriminate against ^{13}C during photosynthesis and preferentially incorporate ^{12}C, the lighter isotope, into biomass. As water stress increases however, isotope discrimination decreases, and ^{13}C is more readily used, increasing the $^{13}C:^{12}C$ in plant tissue. Water stress and the subsequent stomatal closure result in increased $^{13}C:^{12}C$ of tissue relative to non-water-stressed plants, as intracellular ^{12}C is depleted and ^{13}C is used as a reactant in the photosynthesis (Cregg and Zhang, 2000).

Ecological studies have documented decreases in Δ of some coniferous species in response to limited water availability (Aranda et al., 2010; Brandes et al., 2007; Olivas-

Gar

aml

(ca

et a

dec

20

ana

me

ph

W

inc

20

im

cu

dr

Tu

fun

exp

Garcia et al. 2000; Zhang and Cregg, 2005). By definition, Δ of plants grown under ambient CO_2 concentrations is a product of g_s and δC^{13} fixed by Rubisco (carboxylation) and can be used as a surrogate of WUE_i (Cregg and Zhang, 2000; Guehl et al., 1995). Δ is inversely related to WUE_i (A/g_s), because as A increases or g_s decreases, C_i decreases, therefore increasing $\delta^{13}C$ and decreasing Δ (Cregg and Zhang, 2000).

Transpiration efficiency (TE) is the relationship between water used by a plant and the total biomass produced and represents the whole plant water use efficiency. This method takes into account water used in other metabolic processes besides photosynthesis and is calculated as

[biomass produced / water used]

Water-limiting irrigation regimes have been used to increase the TE of a number of plants including *Capsicum annuum* L., *Catharanthus roseus*, and *Olea europaea* (Bacelar et al., 2007; Jaleel et al., 2008; Karam et al., 2009). In a study by Ismail et al. (2007), cyclic irrigation increased TE of tomatoes by 15% compared to single applications.

The response of WUE to drought stress is dependent on plant type, species, cultivar, tree age, provenance, and phenotype, and can be used as a screening process for drought resistance (Adams and Kolb, 2004; Bacelar et al., 2007; Cernusak et al., 2009; Turnbull et al., 2002; Zhang and Cregg, 2005;). In an effort to gain insight into the functional morphology affecting the WUE response, models have been developed, which explain that coniferous evergreens are more resistant to drought stress (increased WUE) than broadleaf trees due to sunken stomata, lignified guard cell walls, and lower pre-dawn osmotic potential (an intrinsic measure of plant resistance to drought).

This study was designed to test the effects of cyclic irrigation regimes on PIP-grown coniferous trees in Northern temperate climates. The overall goal was to develop irrigation guidelines for growers in the upper Midwest that conserve water and reduce leaching of nutrients without sacrificing crop growth. The objectives of this study were to 1) determine effects of cyclic irrigation programs on tree growth, 2) explore underlying physiological mechanisms, and 3) examine the fate of nutrients in response to cyclic irrigation treatments. We also sought to compare various methods of WUE measurements.

Materials and Methods

Site description and experimental design

This study was conducted at the Sandhill site of the Michigan State University Horticulture Teaching and Research Center (HTRC) (lat.: 42.6734°N, long.: 84.4870°E, elev.: 264 m) in Holt, Michigan. This study consisted of two experiments – one using #3 (10.2 L) containers and one using #7 (24.5L) containers. Trees were grown using a pot-in-pot (PIP) system. The soil on site was a well-drained loamy sand (83.1% sand, 8.7% silt, 9.3% clay). Spacing of #3 and #7 containers was 0.5 m and 1 m on-center within rows and between rows, respectively. Rims of the socket pots were approximately 2.5 cm above the ground, and the ground was covered with landscape fabric to control weeds.

Plant materials

In April 2008, 400 plug+2 or 2+2 bare-root transplants (100 of four species; from Peterson's Riverview Nursery, LLC, Allegan, MI) were planted in #3 containers

(GL1200, Nursery Supplies, Inc., Chambersburg, PA) using a 80:20 (volume: volume) mix of pine bark and peat moss (Renewed Earth, Inc., Kalamazoo, MI). Container capacity of the media was 44.5%. The four species used were *Abies fraseri* (Pursh) Poir, *Picea pungens* Engelm. var. *glauca* Regel, *Picea glauca* (Moench) Voss var. *densata*, and *Pinus strobus* L. Also in the spring of 2008, 100 trees grown in #3 containers from a previous study were transplanted into #7 containers (GL2800, Nursery Supplies, Inc.) using the standard media described above.

Irrigation treatments

Daily irrigation depths were based on daily water use estimates of a container-grown conifer in Michigan documented by Warsaw et al. (2009a). Six trees grown in #3 containers of each species were randomly assigned to one of six irrigation regimes. Treatments imposed on trees grown in #3 containers consisted of a combination of daily irrigation depth (1 cm, 2 cm, or 3 cm) and daily cycle frequency (one or four). For trees grown in #3 containers, the 1-1, 2-1, and 3-1 treatments were delivered in a single application, while the 1-4, 2-4, and 3-4 treatments were delivered in four cycles. The 1-1 and 1-4, 2-1 and 2-4, and 3-1 and 3-4 treatments received one, two, and three cm of water daily, respectively. Four trees grown in #7 containers of each species were randomly assigned to one of four irrigation regimes. Treatments imposed on trees grown in #7 containers consisted of a combination of daily irrigation depth (1 cm or 2 cm) and daily cycle frequency (one or four). For trees grown in #7 containers, the 1-1 and 2-1 treatments were delivered in a single application, while the 1-4 and 2-4 treatments were delivered in four cycles. The 1-1 and 1-4 and 2-1 and 2-4 treatments received one and two cm of water daily, respectively.

Once-daily irrigation events occurred at 0600 h daily. Cyclic irrigation treatments were applied at 0600, 1000, 1400, and 1800 h daily and applied 25% of the daily total at each watering. Irrigation was applied from 15 May 2008 to 25 September 2008 and from 21 May to 1 October 2009.

Irrigation was controlled by two timers (Nelson 8014 series Solo Rain); one ran once daily and the other ran four times per day, and volume was controlled via selection of emitter. At the initiation of the studies, water was delivered via non pressure-compensating spray stakes. In July 2008, trees in #3 containers receiving low and medium irrigation rates and all trees in #7 containers were retrofitted with pressure-compensating drip emitters (Netafim, Fresno CA) due to low distribution uniformity within plots. In July 2009, the #7 containers were retrofitted with PCNL pressure-compensating spray stakes (Netafim, Fresno CA) to increase distribution uniformity within pots.

The experimental design was a strip-split plot design with number of cycles as the main plot factor, species as the subplot factor and irrigation rate as the sub subplot factor. All combinations of species x irrigation rate x cycle frequency of trees in #3 and #7 containers were replicated 12 and four times, respectively. Trees were arranged to allow for blocking in time of physiological measurements. Both experiments were surrounded by a row of guard trees. One row on each side of the #7 container plot served as a guard rows, and one row on three sides and three rows on one side of the #3 container plot served as guard rows.

Trees grown in #3 and #7 containers were top-dressed with 60 and 130 grams of 15-9-12 Osmocote® Plus 8-9 month release (The Scotts Co., Marysville, OH), respectively, in the spring of 2008 and 2009. Weeds within containers were controlled through hand-weeding. Pots were turned periodically during the growing seasons to prevent rooting out. In June 2009, squares of landscape fabric impregnated with copper hydroxide (SpinOut®, SePRO, Carmel, IN) were placed between the growing pot and socket pot in the plot of #7 containers. Minimal pruning was performed throughout both seasons.

Growth

Tree growth (caliper and height) was measured monthly, and when possible, measurements were taken approximately 30 days apart. At the beginning of each growing season, prior to bud break, initial tree height was measured, with a standard meterstick, perpendicularly from the rim on the north side of the container to the highest live point of the tree. Average trunk caliper was assessed using a digital caliper. Two caliper measurements were taken perpendicular to each other, north-south oriented and east-west oriented, at a height level with the rim of the container.

Gas exchange

Picea species and *A. fraseri*. Gas exchange was measured periodically in each growing season using a portable gas exchange system (LI-6400, Li-Cor, Lincoln, NE) equipped with a conifer chamber (LI-6400-05, Li-Cor). Mid-day gas exchange was assessed on all trees in #7 containers and on four blocks (96 trees) of #3 containers between 0900 HR and 1700 HR after shoots had fully expanded. One south-facing shoot of the current season's growth was selected from the upper third of the tree and flagged at the beginning of the season so that it could be used in subsequent gas exchange sampling. If necessary, some needles were removed with scissors to ensure that the chamber was adequately sealed. Light-saturated assimilation (A_{max}), stomatal conductance (g_s), and transpiration (E) were measured on clear days using: CO_2 concentration at 400 $\mu mol \cdot mol^{-1}$ and flow of air at 500 $\mu mol \cdot s^{-1}$. To minimize temperature effects during each measurement run, the block temperature was set at the estimated high temperature for the day. Measurements were recorded after the readings had stabilized on the system's real-time graphics screen. At the end of each season, the shoot used for gas exchange was harvested and scanned using a leaf area meter (LI-3000, Li-Cor) to obtain the projected shoot area, by which measurements were adjusted. Sampling dates for #3 and #7 containers were 21 July 2008 and 13 July, 6 August, 25 August, and 2 September 2009 and 22 July 2008 and 24 July, 6 August, 31 August 2009, respectively.

P. strobus. Gas exchange of *P. strobus* was sampled as described previously; however, since the needles were too long to fit in the conifer chamber, a 3x2 cm leaf chamber equipped with a red/blue light-emitting diode light source (Li-6400-02B, Li-Cor) was used to enclose a segment of needles. Two adjacent sun-lit *P. strobus* fascicles (10 needles) from the upper third of the tree were held side-by-side to prevent self-shading and clamped inside the chamber. A_{max}, g_s, and E were measured using optimum conditions within the chamber as described previously and a quantum flux of 1500 $\mu mol \cdot m^{-2} s^{-1}$. Gas exchange measurements were adjusted to reflect actual photosynthetic area by harvesting a randomly selected needle for each tree and determining its radius under a dissecting microscope. Total surface area was calculated assuming each needle represented one-fifth of a cylinder with a length of 3 cm (the length of the leaf chamber).

Foliar analysis

A subsample of sunlit needles was harvested from each tree on 27 July, 2009, dried, and sent to a commercial lab for foliar nutrient analysis (Scotts Inc., Lincoln, NE).

Carbon isotope discrimination

All trees in #7 containers and 144 trees (six blocks) in #3 containers receiving 1-1, 1-4, 3-1, or 3-4 treatments were sampled for carbon isotope discrimination. At the end of the 2009 season, randomly selected branches were harvested, separated by year (2008 and 2009 growth), and dried to a constant weight. Needles were removed and ground with a coffee grinder. At the same time of harvest, stem tissue was sampled using pruners or a chainsaw. A 1-cm disk of the stem was removed at a height equal to the rim of the container (where stem diameter was measured), and 2008 and 2009 growth rings were identified. Stem discs were clamped in a vice, sanded with 150-grit sandpaper to aid in distinguishing growth rings, and outer bark was removed. Rings of trees in #7 containers were separated by year (2008 and 2009) using a hammer and chisel, and tissue was ground with a Wiley mill (General Electric, Fairfield, CT). Rings of trees in #3 containers were sampled by removing 1-2 mg of tissue spanning the width of the ring with a razor blade. Ground foliar tissue (2-3 mg) and wood tissue (1-2 mg) was passed through a #40 sieve and packed into 5x9 mm tin capsules and placed in a 96-well sample tray. Samples were sent to the University of California at Davis Stable Isotope Facility for analysis of the relative abundance of ^{13}C and ^{12}C. The resulting Δ values were expressed relative to international standards PeeDee Belemnite (limestone) (Craig, 1957).

Biomass production

Total biomass was assessed by destructively harvesting a subsample of trees and developing an allometric relationship between caliper and biomass to estimate biomass for those trees not harvested. To develop the allometric relationship, trees within each container size and species were grouped into four size classes based on caliper at the end of the 2009 season. Sixteen trees from each species x size class within each plot were destructively harvested, with the stems cut at substrate level. Tissue was dried to a constant weight, then separated by tissue type i.e. roots, shoots, or needles. Total biomass was determined by summing the mass of the roots, shoots, and needles. A regression analysis was performed between the tree biomass and caliper to estimate biomass for each tree.

Diurnal water potential

Shoot water potential (Ψ) of all *A. fraseri* in #7 containers were measured on two dates during each growing season - once on a day with relatively low VPD and once on a day with relatively high VPD. At two-hour intervals, from pre-dawn to 2000 hours, one fully expanded shoot per tree was harvested and stored in a zippered plastic bag in a cooler of ice until Ψ was determined using a PMS pressure chamber (PMS, Albany, OR). Harvested shoots were stripped of their outer bark and inserted into the pressure chamber's inlet so that approximately 3 cm of the shoot was exposed. The seal was tightened, and pressure was slowly applied until a drop of sap was visible with a handlens. Times of sampling corresponded with gas exchange measurements. Dates of sampling were 31 August and 18 September in 2008 and 16 July and 5 August in 2009.

Substrate moisture measurements

In July 2008, a substrate monitoring system was installed which monitored sixteen trees (eight trees of two blocks) of #7 containers for substrate moisture and temperature. One moisture probe (15 cm long) measuring volumetric water content (VWC) was inserted in each container at a point halfway between the stem and the edge of the container wall at a 45° angle. Probes were connected to a CR1000 datalogger via an AM25T multiplexer (Campbell Scientific, Logan, UT), measuring VWC every 30 seconds, and recording 15-minute averages. Maximum, minimum, and average substrate VWC along with time of maximum and time of minimum were recorded every 15 minutes and every 24 hours. Substrate VWC values were calibrated for organic substrates according to manufacturer's standards. Electrical rain gauge transmitters (Texas Electronics, Inc., Dallas, TX) were installed in two empty #3 containers to estimate precipitation and connected to the datalogger via the multiplexer.

Average leaching fraction of trees grown in #3 containers, sampled from *A. fraseri* and *P. strobus* trees on three dates in 2009 (30 July, 31 July, and 14 August), was 11%. Leaching fraction of trees grown in #7 containers was sampled on two dates in 2009 (31 July and 14 August) and averaged zero.

Nitrate-N, pH, and electrical conductivity in leachate

Leachate was collected from a subsample of two species using the pour-through procedure (Wright et al., 1986; Bilderback, 2001b). *Abies fraseri* and *P. strobus* in four blocks of each experiment were sampled monthly from July to September 2008 and from

May to July 2009. Growing pots were removed from socket pots, media was saturated, and allowed to drain for 30 minutes. Then 0.5 L and 1.0 L of water were applied to each tree in #3 and #7 containers, respectively; leachate was collected in 20-mL vials and stored at 2.5C. Electrical conductivity (EC; Oakton Con110 series, Eutech Instruments, Vernon Hills, IL) and pH (AB15 Accumet basic, Fisher Scientific, Pittsburgh, PA) were measured in the laboratory after collection. Nitrate-N analysis was performed by the MSU Soil and Plant Nutrient Laboratory using flow injection with cadmium reduction (Huffman and Barbarick, 1981). Irrigation water, which was sampled at each collection time, averaged: N03-N, 0.24 ppm; EC, 0.8 dS/M; and pH, 7.8.

Statistical analysis

Data were analyzed using SAS version 9.1 software (SAS Institute Inc., Cary, NC). All variables were tested for normality using PROC UNIVARIATE. PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. Mean separation was performed using Tukey's adjustment. The effects of sampling time for gas exchange, Ψ, nitrate-N, pH, and EC data were analyzed using repeated measures within PROC MIXED. A square root transformation was used to analyze mid-day A_{max} of trees grown in #3 containers, mid-day g_s of trees grown in #7 containers, mid-day NO3-N concentration of leachate collected from trees grown in #7 containers, and leachate EC and foliar N content of trees grown in both size containers. A log transformation was used for the analysis of mid-day WUE_i, diurnal courses of g_s, and biomass production of trees grown in #3 containers. Pearson correlation coefficients for stem caliper, tree height, A_{max}, g_S, canopy temperature, Ψ, Δ, WUE_i, and leachate NO3-N, pH, and EC were determined using PROC CORR.

Results

Weather

Mean maximum daily air temperatures during the growing seasons (1 May to 1 October) were 25.0°C and 23.8°C for 2008 and 2009, respectively. Total precipitation amounts during that time were 462 mm in 2008 and 425 mm in 2009. Total seasonal (1 May to 1 October) reference evapotranspiration (ET_0) exceeded rainfall by 308 mm and 244 mm in 2008 and 2009, respectively (Fig. 3.1). The average minimum and maximum air temperatures during the winter months (1 December 2008 to 31 March 2009), as recorded by the Michigan Automated Weather Network (MAWN), were -7.9°C and 2.0°C (2010).

Substrate moisture

Afternoon irrigation applications increased substrate VWC. The 2 cm depth applied cyclically resulted in greater increases in substrate moisture than the 1 cm depth applied cyclically. There was temporal variation in the magnitude of these fluctuations. The increases in VWC caused by 1000 hr and 1400 hr applications decreased in the beginning of August. Within the season, substrate moisture fluctuations varied greatly (Fig. 3.2).

Water use

Daily water use was estimated from volumetric moisture content data in 2009 (1 May to 1 October). Average daily water use was highest in *P. strobus*, followed by *P. pungens*, *P. glauca*, and *A. fraseri*. Total 2009 seasonal water use of *P. strobus* was nearly twice that of *Picea* species. Mean crop coefficients ranged from 3.4 to 5. On average, crop coefficients were highest in *P. strobus*, followed by *A. fraseri*, *P. glauca*, and *P. pungens*.

Growth

Total height and caliper growth of trees grown in #3 containers varied among species, but was unaffected by irrigation treatment ($p \leq 0.05$; Fig. 3.4). The trend in total mean height and caliper growth during the two-year study was *P. strobus* > *A. fraseri* > *P. pungens* > *P. glauca*.

Biomass production during 2009 and final root:shoot ratios (R:S; needles mass/root mass) of trees grown in #3 containers varied by species ($p \leq 0.05$). Irrigation treatment did not affect biomass accumulation during the 2009 season. The average R:S of *P. pungens* trees was 25% less than that of *P. glauca*, and the mean R:S of *P. strobus* trees was more than twice that of *P. pungens* trees.

Height and caliper growth of trees grown in #7 containers was also species specific. On average, *A. fraseri* trees grown in #7 containers grew tallest, followed by *P. strobus*, *P. glauca*, and *P. pungens* trees. The trend in caliper was similar (*P. strobus* > *A. fraseri* > *P. glauca* > *P. pungens*).

Height and caliper growth of trees grown in #7 containers was greater in year two of the study than year one. Over the two-year production cycle, irrigation regime affected growth of only *P. glauca* and *P. strobus* trees. *Picea pungens* trees receiving the 2 cm irrigation depth had greater ($p \leq 0.01$) caliper growth than *P. pungens* trees receiving the 1 cm depth (Fig. 3.3). *Pinus strobus* trees receiving four cycles per day had greater ($p \leq 0.05$) height growth than *P. strobus* trees receiving only one cycle.

heig

R:S

R:S

was

wa

ac

bi

va

P

c

s

The trend of biomass production of trees grown in #7 containers mirrored that of height growth (*A. fraseri* > *P. strobus* > *P. glauca* > *P. pungens*), and ranking of mean R:S of trees was similar (*P. strobus* > *A. fraseri* > *P. glauca* > *P. pungens*). The average R:S of *P. pungens* was similar to that of *P. glauca*, and the mean R:S of *P. strobus* trees was more than twice that of *A. fraseri* (data not shown).

Biomass production and distribution of *P. pungens* trees grown in #7 containers was affected by irrigation treatment. The 1 cm depth reduced ($p \leq 0.05$) total biomass accumulation and R:S compared to the 2 cm depth. Cycle frequency affected only biomass accumulation of *P. strobus* trees as trees receiving water in four cycles had mean values double that ($p \leq 0.05$) of trees receiving water in one cycle (data not shown).

Physiological responses

Leaf gas exchange

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.

Mid-day gas exchange of single-needle conifers grown in #3 containers varied by species, as well as date of sampling (Table 3.1). Average mid-day A_{max} and g_s values of *Picea* trees were nearly double those of *A. fraseri* trees (Table 3.2). The high irrigation rate increased ($p \leq 0.05$) A_{max} and g_s of *P. pungens* and *P. strobus* trees grown in #3 containers compared to the low rate on one and three dates, respectively. Increasing cycle frequency had no effect on mid-day g_s and decreased ($p \leq 0.05$) A_{max} of *P. glauca* on one date (6 August 2009). There was no effect of irrigation treatment on mid-day gas exchange of *A. fraseri* trees grown in #3 containers on any date.

Mean A_{max} and g_s values of *P. pungens* trees grown in #7 containers were higher ($p \leq 0.05$) than those of *A. fraseri* trees grown in #7 containers (Table 3.2). The response of mid-day gas exchange of trees grown in #7 containers varied depending on sampling date (Table 3.1). Overall, mid-day gas exchange was relatively unresponsive, except for one date (6 August 2009) when *Picea pungens* trees receiving the 2-4 had higher ($p \leq 0.05$) A_{max} and g_s than trees receiving the 2-1 treatment and *P. glauca* trees receiving the cyclic treatment had higher ($p \leq 0.01$) g_s than trees receiving only one cycle. Mid-day gas exchange of *A. fraseri* and *P. strobus* trees grown in #7 containers were unaffected by irrigation treatment on all sampling dates.

Diurnal courses of gas exchange, Ψ, and canopy temperature of trees grown in #7 containers were dependent on day of sampling. The significant cycles x date and level x date interactions in the overall ANOVAs of diurnal g_s and A_{max} were due to changes in rank order of cycles and levels, respectively. On average, A_{max} and g_s were highest at 1000 hr and declined steadily throughout the day. Canopy temperature and Ψ were most extreme at 1600 hr.

There was an effect of sampling date in the overall ANOVA of diurnal g_s and A_{max}. Therefore, data were analyzed separately for each sampling date. Irrigation treatment did not affect diurnal courses of A_{max}, but influenced g_s, Ψ, and canopy temperature. Although g_S and Ψ were not affected at any single sampling time, when pooled across all sampling times within the course of a day to obtain a mean diurnal value, g_S and Ψ were affected by irrigation on one date (5 August 2009) when the high irrigation rate maintained greater ($P \leq 0.05$) g_S and Ψ than the low rate (Fig. 3.4; Fig. 3.5. Both cyclic applications ($p \leq 0.01$) and the high rate ($P \leq 0.05$) reduced canopy temperature throughout the day compared to the once daily application and the low rate, respectively (data not shown).

WUE_i

The ANOVA for mid-day WUE_i (A_{max}/g_S) of conifers grown in #3 containers indicated effects of irrigation regime and date of sampling (Table 3.1). When analyzed by date, WUE_i of all tree species except *A. fraseri* grown in #3 containers responded to irrigation treatment on at least one sampling date. Irrigation rate had a greater effect on WUE_i than did cycle frequency. Most often, trees irrigated with lower rates had higher WUE_i values than trees receiving a higher rate. Although *P. pungens*, *P. glauca*, and *P. strobus* trees responded on at least one date, the effect of irrigation treatment averaged across all dates was not significant in any species (Table 3.2).

Mid-day WUE_i of trees grown in #7 containers varied by species and date of sampling, and on average was unresponsive to irrigation regime (Table 3.1). Cycle frequency did not affect mid-day WUE_i of trees grown in #7 containers, and irrigation rate affected only one species on one date (2 September 2009) when decreasing the irrigation rate increased ($P \leq 0.05$) mid-day WUE_i of *P. strobus* trees. Diurnal courses of WUE_i peaked at 1400 hr, but were unresponsive to irrigation regime.

Integrated water use efficiency

Carbon isotope discrimination values of trees grown in #3 containers varied by species and year of tissue formation. Foliar isotopic composition of 2008 and 2009 wood and foliage of *P. glauca* and *P. pungens* trees grown in #3 containers was similar, but differed in *A. fraseri* ($p \leq 0.05$) and *P. strobus* ($p \leq 0.01$) trees (wood only).

Foliar and wood Δ values of all species of trees grown in #3 containers, except *A. fraseri*, were affected by irrigation regime. The low rate decreased ($p \leq 0.01$) discrimination in year two of the study in *P. glauca* wood and *P. strobus* wood and needles compared to the high rate. Decreasing cycle frequency reduced ($p \leq 0.05$) Δ in *P. glauca* wood in year one of the study and *P. pungens* and *P. strobus* wood in year two of the study.

Foliar Δ was positively correlated ($p \leq 0.05$) with wood Δ in all species in the second year of the study, but was positively correlated ($p \leq 0.05$) with Δ values of wood only in *A. fraseri* trees in the first year of the study. Mid-day leaf g_S was correlated with foliar Δ values from 2008 ($r=0.39$, $p \leq 0.05$) and 2009 ($r=0.43$, $p \leq 0.01$) only in *P. glauca* trees. Δ of foliar and wood tissue from 2009 was positively correlated ($p \leq 0.05$) with height and caliper growth only in *P. pungens*.

The isotopic composition of trees grown in #7 containers also differed by species and age of tissue. Δ of wood ($p \leq 0.05$) and foliar tissue ($p \leq 0.01$) of *P. pungens* trees grown in #7 containers were less in year two than year one. On average, discrimination in wood tissue of *P. strobus* trees and in foliar tissue of *P. glauca* trees was less ($p \leq 0.01$) in year two of the study than year one. Carbon isotopic composition of *A. fraseri* foliage was similar in both years of the study when trees were grown in #7 containers.

The isotopic composition of trees grown in #7 containers was responsive to irrigation regime. The reduced irrigation rate decreased ($p \leq 0.05$) discrimination in *P. pungens* wood in year one, *Picea glauca* trees and *A. fraseri* wood in year two, and *P. strobus* foliage ($p \leq 0.01$) in year two of the study. The only effect of cycle frequency occurred in *P. strobus* wood when trees receiving the 2-4 treatment had higher ($p \leq 0.05$) Δ values than any other treatment (Fig. 3.6).

Foliar Δ values were correlated with Δ values of wood tissue in only *P. pungens* and *A. fraseri* trees in years one, but were correlated in all species in year two. Δ of 2009 foliar tissue was correlated with mid-day A_{max} in all species except *P. glauca.* Δ was correlated with tree growth in *P. pungens* and *A. fraseri* trees.

TE

Water use estimates were not available for trees grown in #3 containers or all trees grown in #7 containers due to logistical and equipment constraints; therefore TE was only calculated for a subset of trees grown in #7 containers. TE was calculated as g biomass produced per kg of water used; water used was determined by summing daily water use (calculated from substrate VWC probes) from 1 May to 30 September 2009. *Pinus strobus* trees were not used in TE analysis due to probe malfunction. Due to the limited number probes measuring soil moisture, the effect of irrigation treatment on TE could not be analyzed by species. TE was not affected by irrigation rate of cycle frequency.

Leachate properties

Mean pH and EC values collected from trees grown in #3 containers was 6.5 and 1.7 dS/M, respectively. Leachate pH, EC, and NO3-N concentrations collected from trees grown in #3 containers were affected by irrigation regime and sampling date. A significant rate x sampling date interaction in pH, EC, and NO3-N occurred in both years of the study. The 2 cm irrigation rate produced leachate with the highest pH and lowest EC in both years of the study. The 3 cm rate decreased NO3-N levels in leachate in both years of the study.

Leachate NO3-N concentration collected from trees grown in #7 containers varied by sampling date also. NO3-N concentrations peaked at the beginning of the season and then declined. NO3-N concentrations were higher ($p \leq 0.01$) in leachate collected from trees receiving the 1 cm rate compared to the 3 cm rate.

Mean leachate pH and EC values collected from trees grown in #7 containers were 6.4 and 1.9 dS/M, respectively. Leachate pH and EC values ranged from 4.7 to 7.5 and 0.5 to 5.0 dS/M, respectively, and varied by date of sample. The only difference in pH as a result of irrigation program was on one sampling date (23 July 2009) when trees receiving the 2 cm rate had higher ($p \leq 0.0001$) mean pH values than trees receiving the 1 cm rate. EC was higher ($p \leq 0.01$) in leachate collected from trees receiving the 1-4 treatment compared to trees receiving the 2-4 treatment.

Plant nutrient status

The 3 cm rate reduced foliar N levels in all species grown in #3 containers compared to the 1 cm rate (Fig. 3.7). Only *P. pungens* and *P. strobus* trees grown in #7 containers and receiving the 2 cm rate had lower foliar N concentrations than trees receiving the 1 cm rate (Fig. 3.8). On average, *Picea* species had the highest foliar N concentrations regardless of the size of container in which they were grown, followed by *A. fraseri* and *P. strobus*.

Discussion

This study was designed to test the effects of cyclic irrigation regimes on PIP-grown coniferous trees in Northern temperate climates. The overall goal was to develop scientifically based irrigation guidelines for growers in the upper Midwest. The objectives of this study were to 1) determine effects of cyclic irrigation programs on the growth of PIP-grown conifers, 2) explore underlying physiological mechanisms governing these growth responses, and 3) examine the fate of nutrients in response to cyclic irrigation treatments. We also sought to compare various methods of evaluating WUE.

Growth responses

Height and caliper response

We hypothesized that cyclic irrigation would increase growth compared to once-daily applications and that less water could be used to achieve this growth. Counter to our hypothesis, tree height growth in three of the four species investigated was similar, regardless of the number of cycles in which water was applied; however, we did observe increases in height growth of one species, *P. strobus*, with cyclic irrigation. This growth increase due to increasing cycle frequency is consistent with previous studies testing cyclic irrigation on containerized woody species (Beeson, 1992; Beeson and Haydu, 1995; Fain et al., 1999; Ruter, 1998; Witmer, 2000).

Past studies testing cyclic irrigation have predominantly included broadleaf plants, not conifers, and have been performed in the southeastern U.S., where environmental conditions are much different than those in the upper Midwest. Lower seasonal and mid-day temperatures and a shorter growing season in Northern climates may mean that container-grown trees are subjected to less stress than similar specimens grown in the Southwest. Therefore, increases in growth resulting from cyclic irrigation are more subtle in the upper Midwest than in the Southwest.

Increases in tree height, the standard by which coniferous evergreen trees are sold (ANLA, 2004), was similar regardless of irrigation rate. This is in agreement with numerous studies which have demonstrated equal or superior growth rates of woody ornamental crops at reduced irrigation volumes (Beeson and Haydu, 1995; Groves et al., 1998; Martin et al., 1989; Roberts and Schnipke, 1987; Warsaw et al., 2009a; Warsaw et al., 2009b).

Contrasting responses of growth parameters were also observed. Stem radial growth was more responsive to irrigation treatment than height growth. Height growth is largely determined during bud formation in the previous year and is completed relatively early in the season whereas radial growth continues throughout the growing season. Therefore, height growth is not subject to environmental stresses such as inadequate water availability and supra-optimal temperatures, which are common in mid to late summer.

Observations of larger, fast-growing container-grown plants being more responsive to cyclic irrigation than those that are smaller and slow-growing have also been documented (Beeson and Haydu, 1995; Witmer, 2000). Moreover, species possessing the ability to adapt to drought stress also have an advantage in maintaining growth under reduced water availability; however, some responses, like increased R:S may be limited by the restricted media volume of containers. We hypothesized that the growth response to cyclic irrigation would be greater in 1) relatively fast-growing trees like *P. strobus* and *P. pungens* and 2) year two of the study than in year one.

We expected tree growth to be more reflective of the imposed treatments in year two of the study than in year one; however, in 2009 Michigan experienced its sixth coolest summer on record since 1895. More importantly, the period from June to August 2009 was the fifth coolest ever recorded for this period (NOAA, 2009). Additionally, an excess of 87 mm of precipitation fell from 15 June to 15 August 2009 compared to the same period in 2008. The mild conditions of 2009 may have been a factor in the general unresponsiveness to cyclic treatments in the second year of the study. In 2008, growth of trees in #7 containers was more responsive to irrigation treatment than in 2009. During 2008, trees may also have been undergoing transplant stress from initial planting in May of that year. Our results suggest that cyclic irrigation may be useful in reducing plant water stress during establishment.

Physiological responses

Previous studies testing cyclic irrigation on broadleaf woody ornamentals have attributed the resulting increase in growth to cumulative reductions in mid-day water stress (Beeson, 1992; Witmer, 2000) and subsequent prevention of stomatal closure (greater g_S), therefore increasing net photosynthesis (Beeson, 1992; Witmer, 2000). Based on this theory, we expected that applying a pulse of irrigation in the afternoon would dampen the mid-day fluctuations of gas exchange; however, most often we obtained similar mid-day g_S values regardless of irrigation cycle frequency. The sampling date on which increasing cycle frequency increased mid-day g_S was preceded by four days without precipitation, whereas the other sampling dates were preceded by at most two rain-free days. This indicates that cyclic irrigation may only be effective in maintaining mid-day g_S under hotter and drier conditions than were typical in 2009.

Empirical models describing g_S as a function of soil moisture availability predict that conifers are more drought tolerant than broadleaf trees and shrubs due to their lower osmotic potential, sunken stomata, and lignified guard cell walls (Gao et al., 2002). This may explain the relative unresponsiveness of conifers in our study to cyclic water applications compared to broadleaf trees and shrubs in previous studies.

The increases in mid-day A_{max} and g_S of *P. strobus* trees grown in #3 containers due to increased substrate moisture due to higher irrigation rates is similar to observations of mature *P. strobus* forest trees under mesic and drought conditions (Maier and Teskey, 1992). Reductions in caliper growth of *P. pungens* trees grown in #7 containers caused by the decreased irrigation rate was not accompanied by reductions in mid-day A_{max} and both *P. pungens* and *P. strobus* trees grown in #3 containers had lower mid-day A_{max} caused by the reduced irrigation rate without concomitant reductions in growth. This decoupling of gas exchange and growth suggests that other factors besides A_{max} were limiting growth.

Mid-day Ψ, a measure of plant water status, is often of interest when testing cyclic irrigation. High Ψ is not necessarily a result of well-watered, non-stressed trees, but can also be caused by partially closed stomata in response to drought stress (Reich and Hinckley, 1989); however, the concurrent higher g_S and Ψ due to the high irrigation rate that we observed in our study indicate that trees receiving the high irrigation rate were less stressed than those receiving the low rate. The consistency of Ψ across all cycle frequencies in our study differs from past studies testing cyclic irrigation on container-grown broadleaf trees (Beeson, 1992; Beeson and Haydu, 1995). During the nighttime, plant water status equilibrates with that of the substrate; as such, pre-dawn values of Ψ are assumed to be equivalent to those of the substrate. On two of the three days sampled, we found differences in pre-dawn Ψ between plants irrigated with the high rate and those receiving the low rate. This is similar to observations of two-year-old *P. ponderosa* seedlings under water-stressed and well-watered conditions (Olivas-Garcia et al., 2000). Furthermore, the reductions in mean diurnal Ψ and g_S of *A. fraseri* trees caused by the reduced irrigation rate mimics the effects of high root-zone temperatures on *A. rubrum* trees documented by Graves et al. (1989).

The effect of irrigation regimes on WUE_i was highly dependent on environmental conditions. The parameter's inconsistent response demonstrates the method's limitations as an accurate predictor of WUE. Within the #3 containers, the species with the highest WUE_i (*P. strobus*) was most capable of handling water stress and therefore did not experience large reductions in growth as a result of water-limiting irrigation regimes. We were unable to link the growth decline of *P. strobus* trees in #7 containers as a result of irrigation cycle frequency to reductions in mid-day gas exchange. Increases of WUE_i preceding 1400 hr confirm the occurrence of mid-day reductions in g_s relative to A_{max} (Beeson, 1992).

WUE_i and Δ have been correlated in a number of species (Roussel et al. 2009); however, we did not find significant relationships between these parameters in most of the species tested. This might be due to the fact that WUE_i represents gas exchange of sun-lit foliage on clear days and does not take into account gas exchange of shaded foliage, the effect of cloudy days, or plant respiration.

The increases in Δ that we observed in response to cycle frequency indicate that the cyclic treatments created improved the water status in some species. Foliar N can be used as a surrogate for carboxylation capacity (Adams and Kolb, 2004; Field and Mooney, 1986). Regardless of container size in which trees were grown, leaf and wood Δ values of trees receiving the high irrigation rate were equal to or exceeded those of trees receiving the low irrigation rate, while foliar N concentrations were similar to or were reduced by the higher irrigation rate. These contrasting responses indicate that reduced carboxylation capacity due to reduced foliar N at high irrigation rates was not a factor affecting Δ of trees. Thus, it was thought that g_s must be limiting assimilation of ^{13}C into plant tissue.

The relationship between WUE_i and Δ is stronger than that of g_s and Δ and A_{max} and Δ, because the drawdown of C_i is actually a function of $A_{max}:g_s$. Concomitant re

ar

W

o

d

t

F

2

b

a

g

(

reductions of mean mid-day g_S and Δ were only observed in *P. strobus* year two wood and foliage from trees grown in #3 containers at the low rate compared to the high rate. WUE_i is highly variable as it is a function of the relationship between A and g_S and is only a snapshot of plant functions at one point in time. Δ is a more accurate and sensitive depiction of plant stress, because the signature is incorporated over the entire duration of tissue formation.

Plant carbon pools differ in their isotopic compositions relative to leaves (Bowling et al., 2008). We observed the well-documented ^{13}C enrichment of wood tissue relative to bulk leaf matter, indicating postphotosynthetic carbon isotope fractionation (Brandes et al., 2006; Damesin and Lelarge, 2003; Helle and Schleser, 2004). Similar to height growth, needle composition is highly dependent on plant status at time of bud formation during the previous year and growing conditions during needle expansion of the current year. Δ of wood tissue is considered a more accurate representation of plant stress than that of bulk leaf tissue, because the isotopic signal of annual rings is integrated over the duration of the current growing season.

Leachate and nutrient responses

Electrical conductivity levels were within the recommended range of 0.2 and 0.5 dS/M for controlled release fertilizers (Bilderback, 2001b). Theoretically, soluble salts will accumulate faster in containers receiving low irrigation volumes, as with the low rate and/or cyclic regimes, than containers receiving high irrigation amounts. We observed similar EC values regardless of irrigation cycle frequency. In #3 containers, the reduction in soluble salts at the 3 cm rate was likely due to increased leaching of nutrients and was confirmed visually by chlorosis of *P. strobus* needles during the second growing season. Substrate pH was rarely within the recommended range of 5 to 6 (Bilderback, 2001b). This is likely a result of the alkaline water (average 7.8), typical of Michigan groundwater, which was applied to trees.

Nitrate-N levels in leachate exceeded the recommended 15 to 25 ppm (Bilderback, 2001b) for all treatments. We did not observe the previously documented reductions in leachate NO3-N concentrations as a result of cycle frequency (Fare et al., 1996; Tyler et al. 1996a); however, decreased leachate NO3-N concentrations were observed in trees receiving the high rate versus the low rate. These results are consistent with findings of previous studies (Tyler et al., 1996b; Warsaw et al., 2009b).

The reason for the differences in early season NO3-N concentrations between years is unclear. It is unlikely that residual nutrients from the previous year played a part, considering the low September 2008 concentrations caused by the high irrigation rate. Most likely, differences in temperature affected the release rate early in the season and high precipitation increased leaching of NO3-N during the second season compared to the first season.

Plant nutrient status

An often cited benefit of cyclic irrigation is increased nutrient efficacy and subsequent increases in foliar N due to reduced leaching. We found nutrient retention to be a function of reduced irrigation rate, not increased cycle frequency. Reduced foliar N levels of *P. pungens* and *P. strobus* trees grown in #7 containers at the 2 cm rate, representing a typical application, indicate that growers may be leaching nutrients and sacrificing nutrient efficacy using this application rate.

We did not observe the previously documented (Reich et al, 1998) increases in A_{max} with rising foliar N concentrations. In fact, the only significant correlation between A_{max} and foliar N was when the two parameters were inversely related in *P. strobus* trees grown in #3 containers. In this case, water limitations associated with the low irrigation rate increased foliar N concentrations by retaining nutrients in the substrate, but restricted A_{max}.

Conclusion

Cyclic irrigation increased growth only of container-grown *P. strobus* trees. It is likely that the beneficial effects of cyclic irrigation were underestimated, due to the mild summer of 2009. Reducing irrigation rates by 50% is a viable method of water conservation for container nurseries in the upper Midwest. The low irrigation rate was enough to promote reductions in caliper growth only of *P. pungens* trees, regardless of the size of container in which they were grown, suggesting that this rate may be sufficient for the other species tested. Reduced foliar N levels and increases in leachate NO3-N concentration at the 2 cm rate, representing a typical application, indicate that growers are leaching nutrients and sacrificing nutrient efficacy by using this application rate. However, the decreases in growth of some species (*P. pungens*) at reduced irrigation rates suggests that in some cases growers must choose between increased nutrient retention and growth optimization. Our findings confirm that carbon isotope composition of wood tissue is a sensitive and more accurate representation of WUE than WUE_i.

Table 3.1: Summary repeated measures analysis of variance (ANOVA) for mid-day photosynthesis (A_{max}), leaf stomatal conductance (g_s), and WUE_i (A_{max}/g_s) of single-needle conifer species (*P. glauca* var. densata, *P. pungens*, and *A. fraseri*) grown in #3 or #7 containers in a PIP production system under varying combinations of irrigation rate and cycle frequencies.

	F-values							
	#3 containers				#7 containers			
Source of Variation	d.f.	A_{max}	g_s	WUE_i	d.f.	A_{max}	g_s	WUE_i
Between subjects								
Species (Spp)	2	91.33***	59.56***	4.64	2	15.46**	15.86**	2.98
Cycles	1	7.17	0.11	1.65	1	3.24	10.37*	7.2
Spp*Cycles	2	0.1	0.82	3.56	2	11.63**	15.02**	2.2
Rate	2	4.85**	2.99	2.3	1	4.19*	6.39*	3.84
Spp*Rate	4	1.37	0.61	0.49	2	4.12*	4.55*	4.98*
Cycles*Rate	2	0.27	0.22	0.61	1	0.01	1.03	5.72*
Spp*Cycles*Rate	4	1.48	1.75	2.65*	2	4.21*	6.57**	7.42**
Within subjects								
Date	3	16.24***	78.25***	90.24***	3	115.12***	161.12***	25.39***
Date*Spp	6	5.89***	8.41***	3.59**	6	12.44***	12.96***	3.49**
Date*Cycles	3	2.18	7.18***	14.78***	3	16.44***	15.18***	10.63***
Date*Rate	6	3.35**	6.47***	1.36	3	2.13	1.36	0.09
Date*Spp*Rate	12	1.03	1.39	0.69	6	2.04	2.34*	0.31
Date*Spp*Cycles	6	0.78	1.2	0.95	6	3.84**	3.98**	0.56
Date*Cycles*Rate	6	1.21	1.13	1.19	3	0.63	0.59	0.17
Date*Spp*Cycles*Rate	12	0.97	1.69	0.41	6	1.42	1.42	0.45

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

Table 3.2: 2009 mid-day net photosynthetic rate (A_{max}; µmol·m^{-2}·s^{-1}) and leaf stomatal conductance (g_S; mol H_2O·m^{-2}·s^{-1}), and instantaneous water use efficiency (WUE_i; A_{max}/g_S) of four species of coniferous trees grown in #3 or #7 containers in a PIP production system and receiving three or two irrigation rates, respectively. Cycle frequency x rate interactions were not significant within any species, therefore data was averaged across cycle frequencies and all sampling dates. Means with different letters are significantly different at $p \leq 0.05$. Within species differences are represented by lowercase letters. Overall differences are represented by uppercase letters.

	#3 containers					#7 containers				
	Rate (cm)	*P. glauca*	*P. pungens*	*A. fraseri*	*P. strobus*	Rate (cm)	*P. glauca*	*P. pungens*	*A. fraseri*	*P. strobus*
A_{max}	1	18.30	15.09	8.56	7.20a	1	13.26a	14.60	10.69	10.03
	2	17.92	14.62	9.56	10.55ab	2	16.05b	15.98	10.52	10.61
	3	18.07	16.99	9.90	12.27b	Overall	14.72A	15.29A	10.60B	10.33
	Overall	18.10A	15.59A	9.30B	9.74					
g_S	1	0.27	0.24	0.11	0.06a	1	0.20a	0.29	0.18	0.09
	2	0.29	0.26	0.14	0.10ab	2	0.28b	0.35	0.17	0.11
	3	0.31	0.28	0.14	0.11b	Overall	0.24A	0.32A	0.17B	0.10
	Overall	0.29A	0.26A	0.13B	0.09					
WUE_i	1	73.09	76.73	83.66	113.27	1	69.50	58.36	63.99	111.88
	2	66.12	69.99	83.27	116.99	2	63.54	53.04	67.52	117.13
	3	65.56	65.71	81.40	113.19	Overall	66.39A	55.70A	65.83A	114.62B
	Overall	68.23A	70.65AB	82.83B	114.51C					

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

Figure 3.1: Weekly rainfall and reference evapotranspiration (ET_0) from Michigan Automated Weather Network at Michigan State University (MSU) Hancock Turfgrass Research Center (2008) and MSU Horticulture Teaching and Research Center (2009), East Lansing, MI. Rainfall deficit = ET_0 – rainfall.

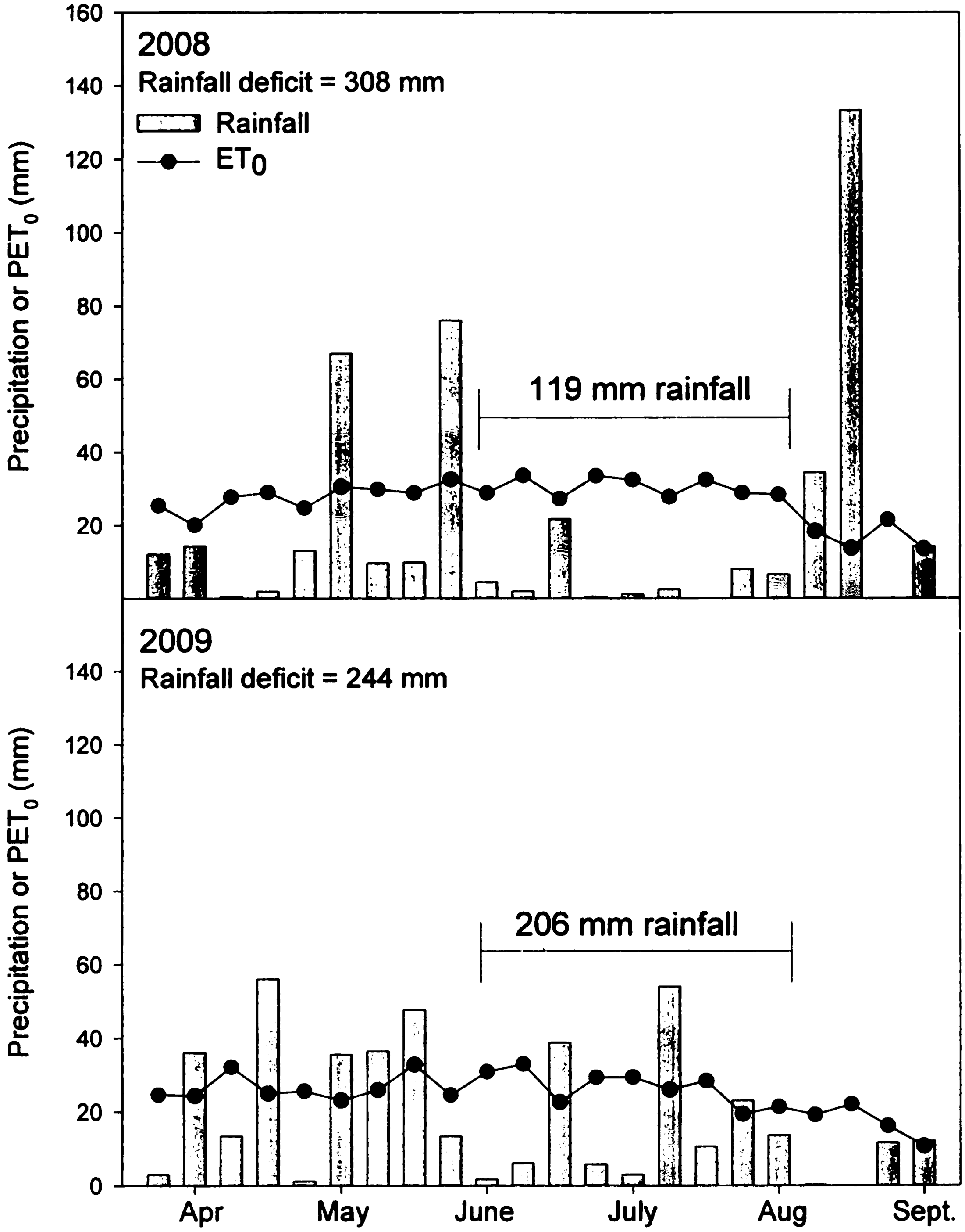


Figure 3.2: Container capacity and seasonal course of substrate volumetric moisture content (VWC; %) of a subsample of 16 coniferous trees grown in #7 containers in a PIP facility under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (one or four).

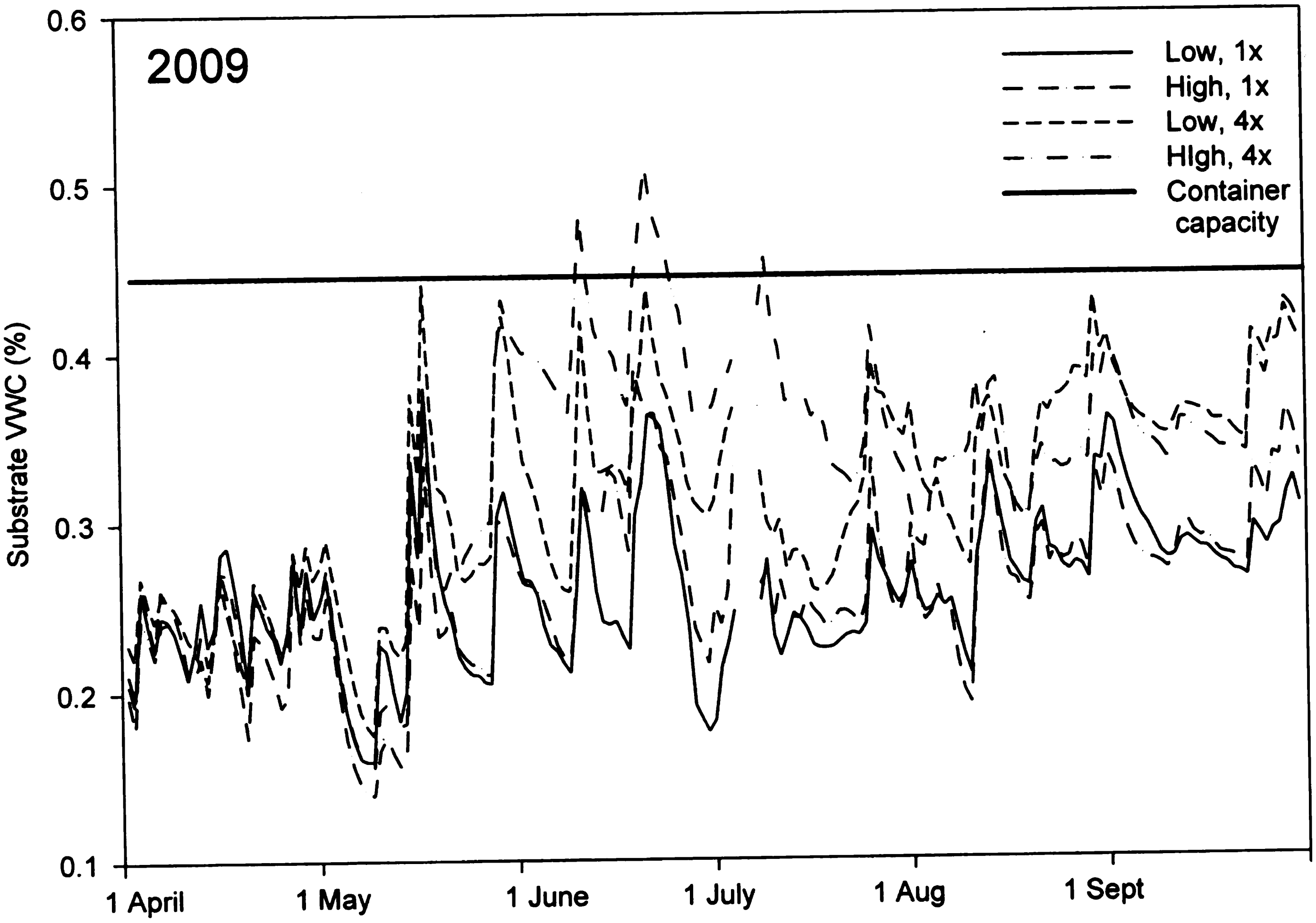


Figure 3.3: Stem radial growth (±SE) over the two-year production cycle of four coniferous tree species grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (one or four). Effect of cycles was not significant; data averaged across both cycle frequencies (n=8). Means with different letters are statistically different at p≤0.05.

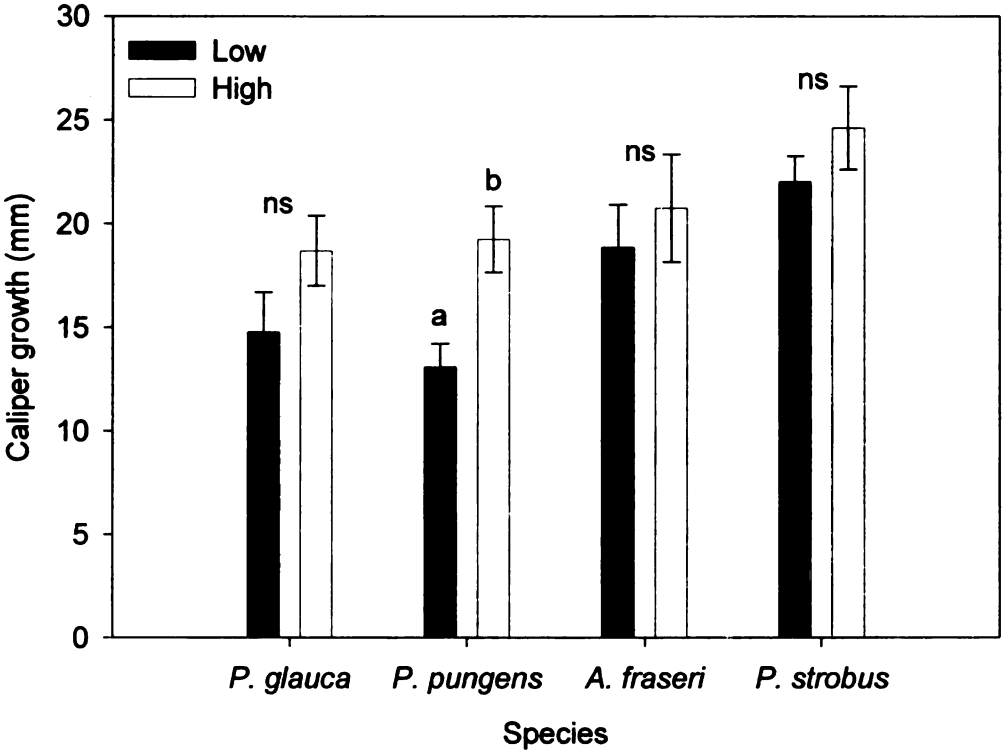


Figure 3.4: Diurnal course of mean leaf stomatal conductance (g_S; mol H2O•m^{-2}•s^{-1}) and (±SE) of *A. fraseri* trees grown in #7 containers in a PIP production system under two irrigation rates (1, 2, or 3 cm) on three dates. Cycles x rate interactions were not significant, therefore data was averaged across cycles. The high irrigation rate increased ($p \leq 0.05$) mean diurnal g_S only on 5 August 2009 ($\alpha = 0.05$; n=4).

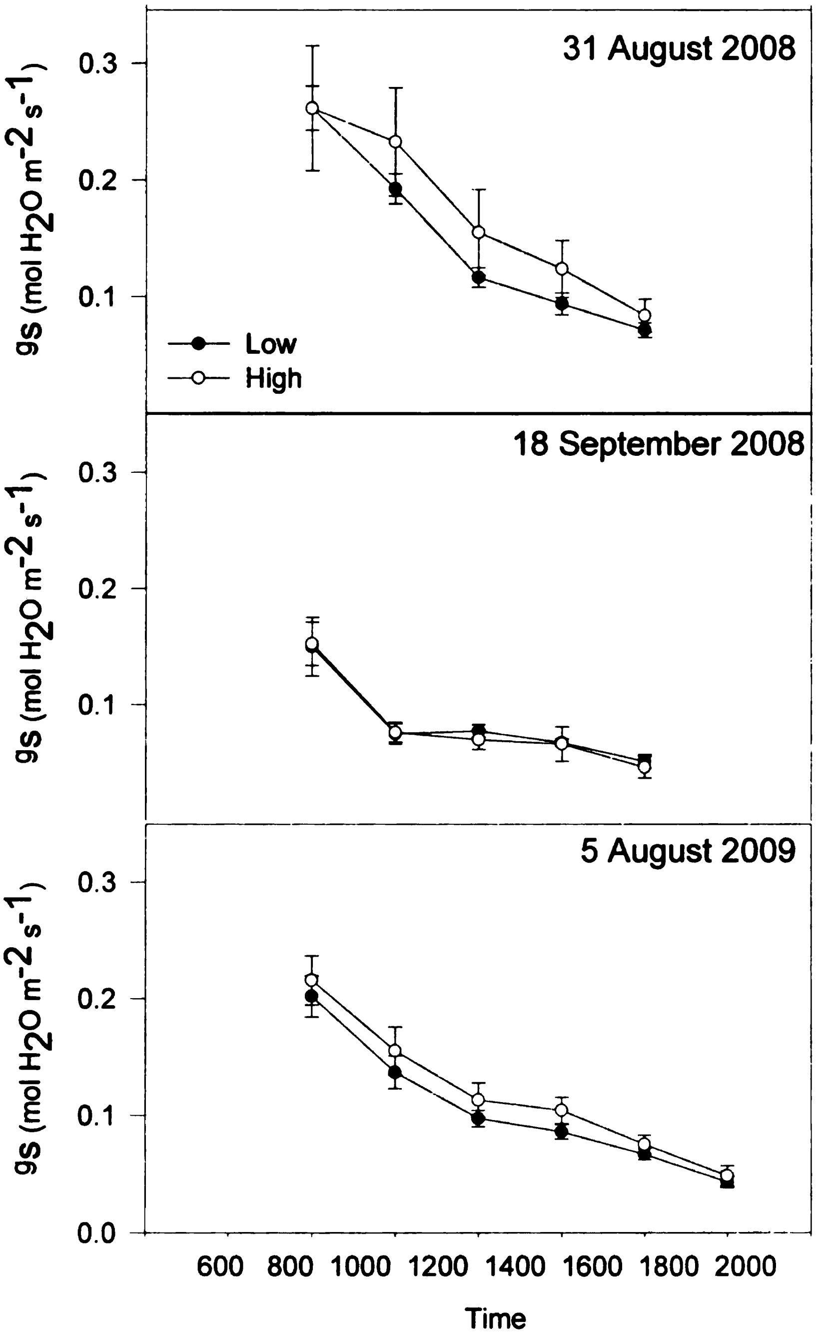


Figure 3.5: Diurnal course of shoot water potential (MPa) (±SE) of *A. fraseri* trees grown in #7 containers in a PIP production system under two irrigation rates (1 or 2 cm) (α=0.05; n=8) on three dates. Cycle frequency x level interaction was not significant, therefore data was averaged across cycles. * indicates significance of mean daily shoot water potential at $p \leq 0.05$.

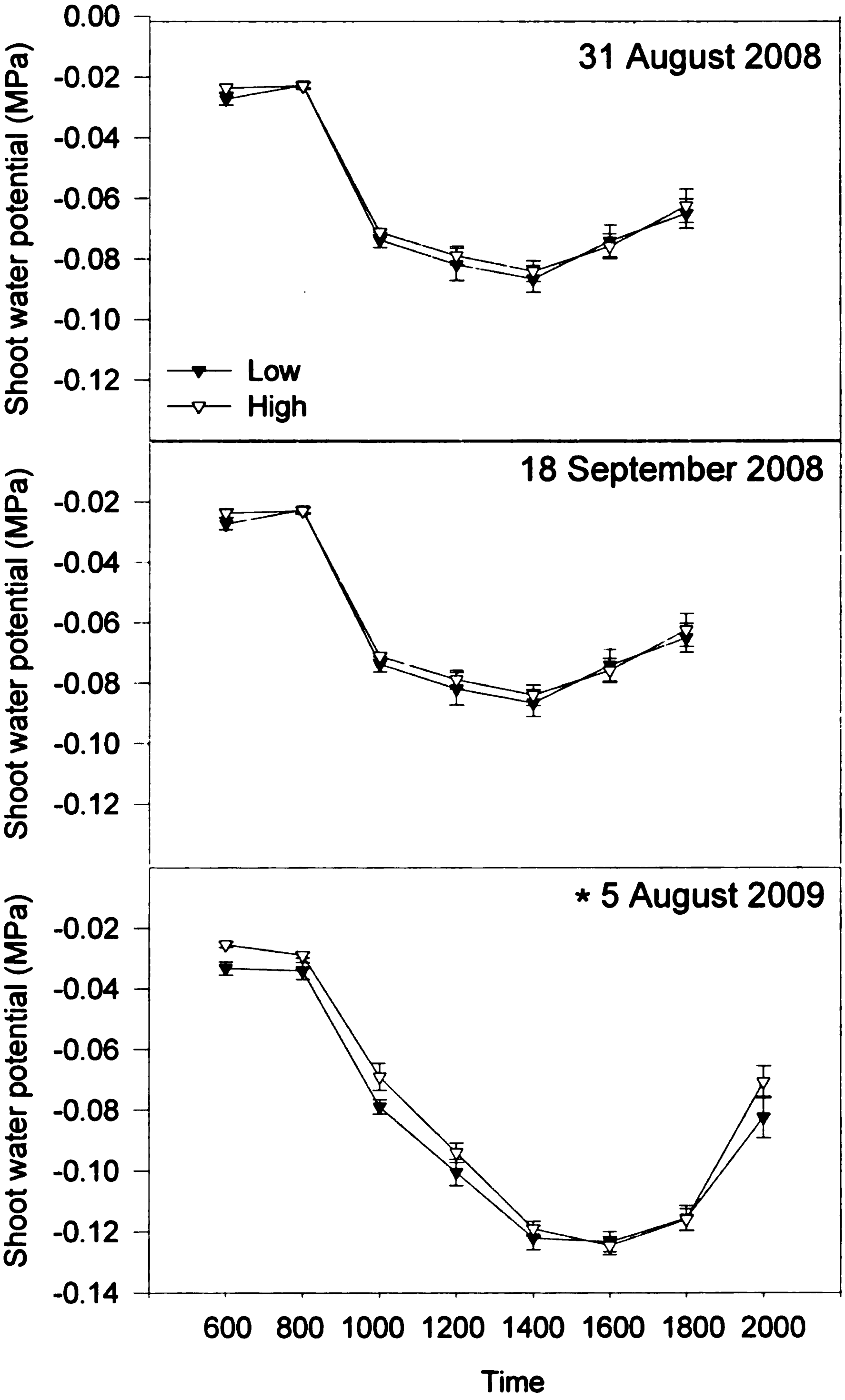


Figure 3.6: Mean (±SE) Δ values of 2008 and 2009 wood of four coniferous tree species grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (1 or 4) (α=0.05; n=4).

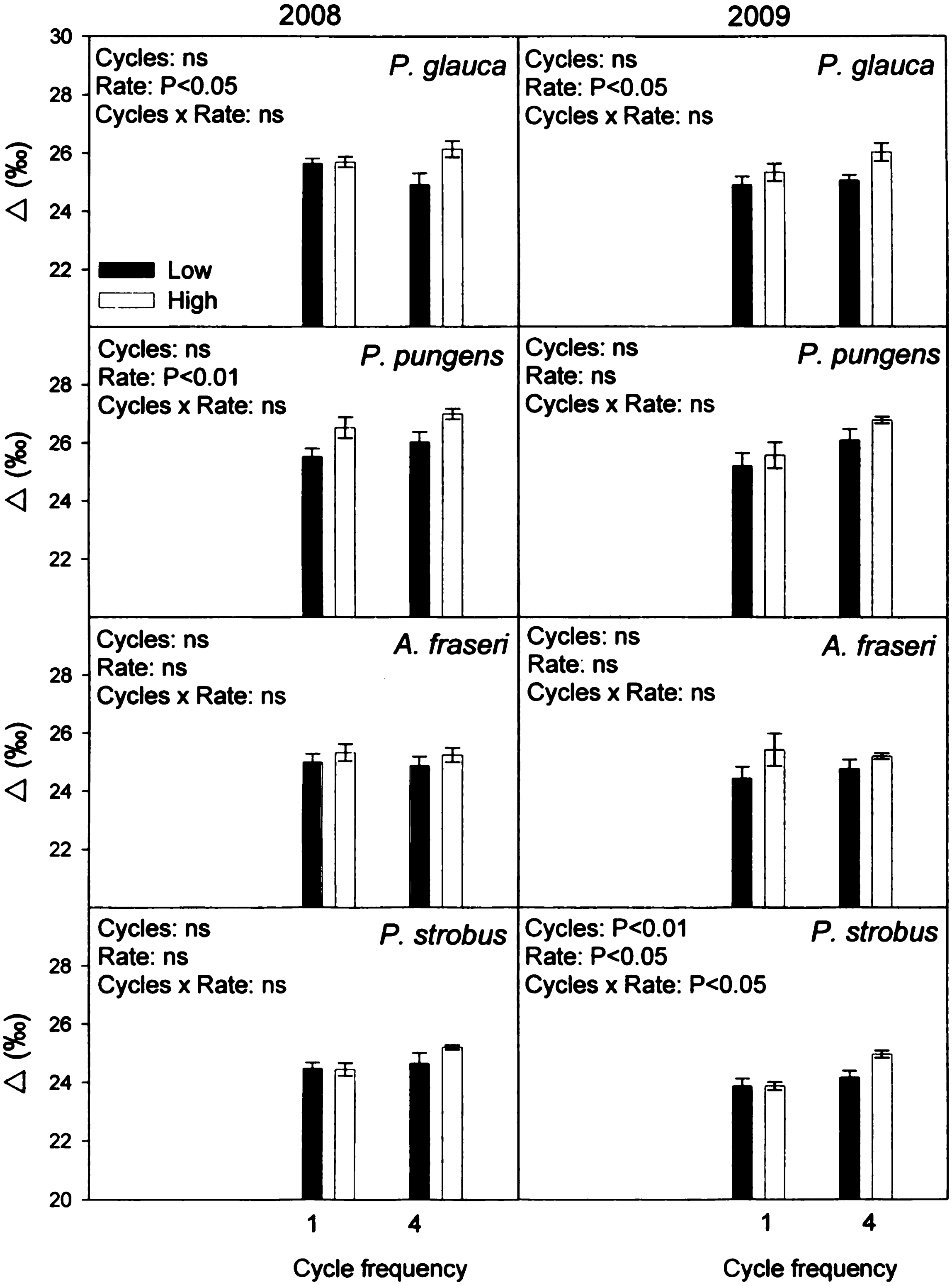


Figure 3.7: End of season mean 2009 N concentration (%; ±SE) of foliar samples collected in 2009 four coniferous tree species grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1, 2, or 3 cm) and daily cycle frequencies (1 or 4). Effect of cycles was not significant; data averaged across both cycle frequencies (n=8). Means with different letters are statistically different at p≤0.05.

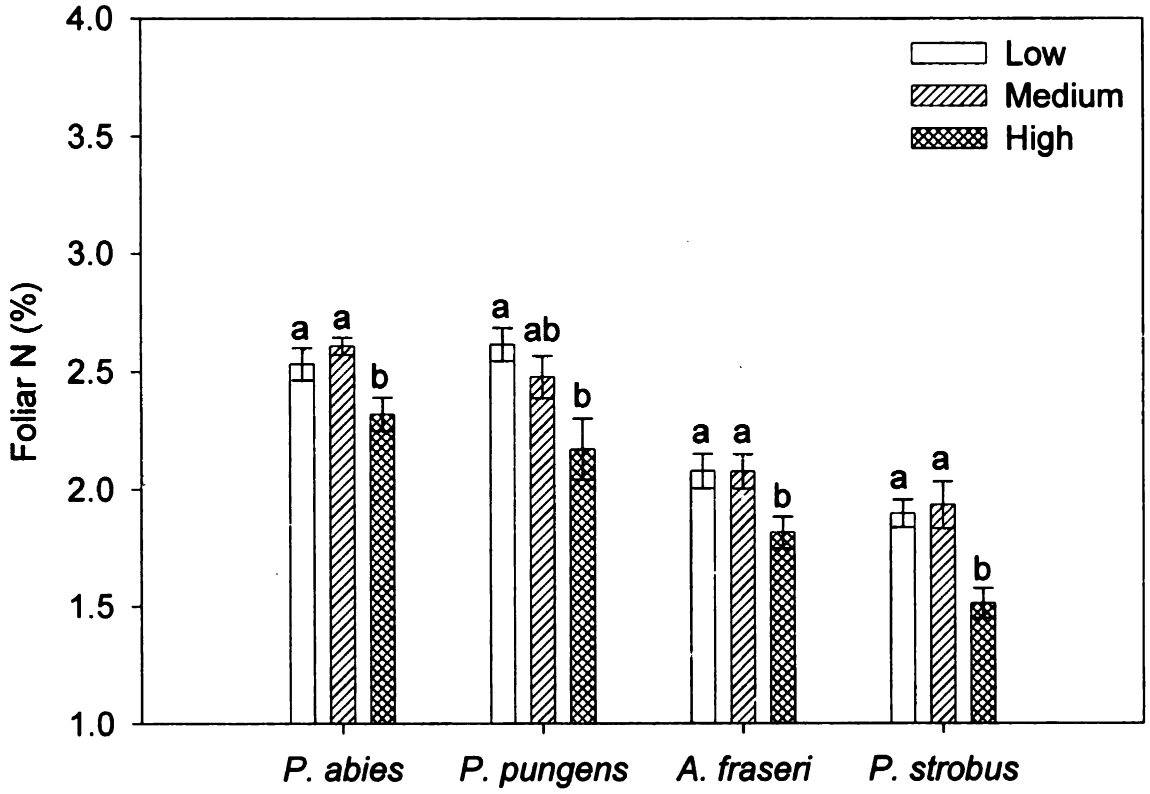
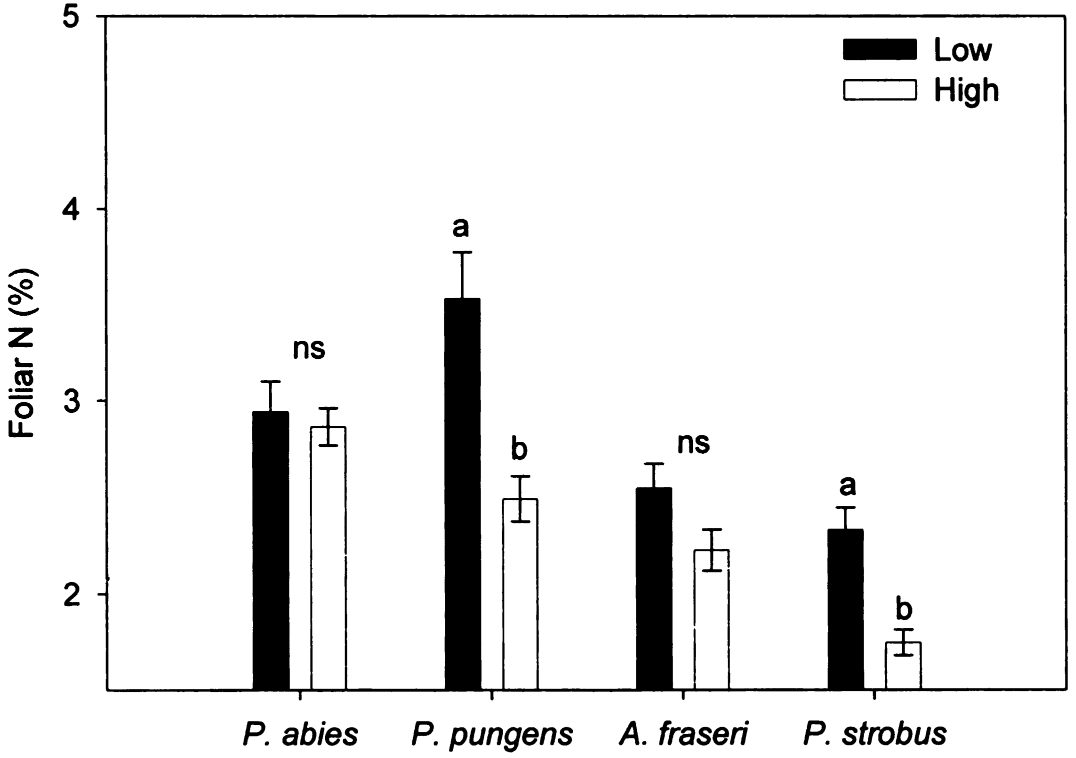


Figure 3.8: End of season 2009 N concentration (%; ±SE) of foliar samples from four coniferous tree species grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (1 or 4). Effect of cycles was not significant; data averaged across both cycle frequencies (n=8). Means with different letters are statistically different at p≤0.05, ns=not significant.



LITERATURE CITED

Adams, H. D. and T. E. Kolb. 2004. Drought responses of conifers in ecotone forests of northern Arizona: tree ring growth and leaf 13C. Oecologia 140: 217-225.

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

Anyia, A.O. and H. Herzog. 2004. Water use efficiency, leaf area, and leaf gas exchange of cowpeas under mid-season drought. Europ. J. Agronomy 20:327-339.

Aranda, I. R. Alia, U. Ortega, A.K. Dantas, and J. Majada. 2010. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* seedlings. Tree Genetics and Genomes 6:169-178.

Bacelar, E.A., Moutinho-Pereira, J.M., Goncalves, B.C., Ferreira, H.F., and C.M. Correia. 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environmental and Experimental Botany 60:183–192.

Bauerle, W. L., W. W. Inman, and J.B. Dudley. 2006. Leaf abscisic acid accumulation in response to substrate water content: Linking leaf gas exchange regulation with leaf abscisic acid concentration. Journal of the American Society for Horticultural Science 131(2): 295-301.

Beeson, Jr., R.C. 1992. Restricting overhead irrigation to dawn limits growth in container-grown woody ornamentals. Hortscience 27(9): 996-999.

Beeson, Jr., R. C. and J. Haydu. 1995. Cyclic microirrigation in container-grown landscape plants improves plant growth and water conservation. Journal of Environmental Horticulture 13(1): 6-11.

Beeson, Jr., R. C., M. A. Arnold, T.E. Bilderback, B. Bolusky, S. Chandler, H.M. Gramling, J.D. Lea-Cox, J. R. Harris P.J. Klinger, H.M. Mathers, J.M. Ruter, and T.H. Yeager. 2004. Strategic vision of container nursery irrigation in the next ten years. Journal of Environmental Horticulture 22(2): 113-115.

Bilderback, T.E. 2001a. Environmentally compatible container plant production practices. Proc. Int. Symp. on Growing Media & Hydroponics. Acta Hort. 548. International Society of Horticultural Science.

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

Bowling, D.R. D.E. Pataki, and J.T. Randerson. 2008. Carbon isotopes in terrestrial ecosystem pools and CO2 fluxes. New Phytologist 178:24-40.

Brandes, E., N. Kodama, K. Whittaker, C. Weston, H. Rennenberg, C. Keitel, M.A. Adams, and A. Gessler. 2006. Short-term variation in the isotopic composition of organic matter allocated from the leaves to the stem of *Pinus sylvestris*: effects of photosynthetic and postphotosynthetic carbon isotope fractionation. Global Change Biology 12:1922-1939.

Brandes, E., J. Wenninger, P. Koeniger, D. Schindler, H. Rennenberg, C. Leibundgut, H. Mayer, and A. Gessler. 2007. Assessing environmental and physiological controls over water relations in a Scots pine (*Pinus sylvestris* L.)stand through analyses of stable isotopic composition of organic matter. Plant, Cell and Environment 30:113-127.

Bsoul, E., R. St Hilaire, and D.M. VanLeeuwen. 2007. Bigtooth maples from selected provenances effectively endure deficit irrigation. Hortscience 42(5): 1167-1173.

Cernusak, L. A., K. Winter, and B.L. Turner. 2009. Physiological and isotopic (delta13C and delta18O) responses of three tropical tree species to water and nutrient availability. Plant, Cell and Environment 32(10): 1441-1455.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica Et Cosmochimica Acta 12(1-2): 133-149.

Cregg, B. and J. Zhang. 2000. Carbon isotope discrimination as a tool to screen for improved drought tolerance. Proceedings of the 11th Metropolitan Tree Improvement Alliance (METRIA) Conference, Gresham, OR.

Damesin, C. and C. Lelarge. 2003. Carbon isotope composition of current-year shoots from *Fagus sylvatica* in relation to growth, respiration and use of reserves. Plant, Cell and Environment 26(2):207-219.

Fain, G. B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1999. Cyclic irrigation improves irrigation application efficiency and growth of sawtooth oak. Journal of Arboriculture 25(4): 200-203.

Fare, D. C., C.H. Gilliam, G.J. Keever, and R.B. Reed. 1996. Cyclic irrigation and media affect container leachate and ageratum growth. Journal of Environmental Horticulture 14(1): 17-21.

Field C. and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish TJ (ed) On the economy of plant form and function. Cambridge University Press, London, p 25–55.

Gao, Q., P. Zhao, X. Zeng, X. Cai, and W. Shen. 2002. A model of stomatal conductance to quantify the relationship between leaf transpiration, microclimate and soil water stress. Plant, Cell and Environment 25: 1373-1381.

Graves, W. R., M. N. Dana, and R.J. Joly. 1989. Root-zone temperature affects water status and growth of red maple. Journal of the American Society for Horticultural Science 114(3): 406-410.

Groves, K. M., S. L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizers: I. Effect on plant growth and mineral nutrient content in containerized plant production. Journal of Environmental Horticulture 16(3): 176-181.

Guehl, J.M., C. Fort, and A. Ferhp. 1995. Differential response of leaf conductance, carbon isotope discrimination and water use efficiency to nitrogen deficiency in maritime pine and pedunculate oak plants. New Phytologist 131:149-157.

Hand, M. J., E. Young, and A.C. Vanconcelos. 1982. Leaf water potential, stomatal resistance, and photosynthetic response to water stress in peach seedlings. Plant Physiology 69: 1051-1054.

Haydu, J. J. and R. C. Beeson, Jr., R.C. 1997. Economic feasibility of micro-irrigating container-grown landscape plants. Journal of Environmental Horticulture 15(1): 23-29.

Helle, G. and G.H. Schleser, 2004. Beyond CO2-fixation by Rubisco – an interpretation of 13C/12C variations in tree rings from novel intra-seasonal studies on broad-leaf trees. Plant, Cell, and Environment 27, 367–380.

Huffman, S.A. and K.A. Barbarick. 1981. Communications in soil science and plant analysis. 12(1):79-89.

Ismail, S.M., K. Ozawa, and N.A. Khondaker. 2007. Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. First Power). Agricultural Water Management 95(2008): 116-122.

Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam, and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. Comptes Rendus Biologies 331(1): 42-47.

Karam, F., R. Masaad, R. Bachour, C.Rhayem, and Y. Rouphael. 2009. Water and radiation use efficiencies in drip-irrigated pepper (*Capsicum annuum* L.): response to full and deficit irrigation regimes. European Journal of Horticultural Science 74(2): 79-85.

Keever, G. J. and G. S. Cobb. 1985. Irrigation scheduling effects on container media and canopy temperatures and growth of 'Hershey Red' azalea. Hortscience 20(5): 921-923.

Maier, C.A., and R.O. Teskey. 1992. Internal and external control of net photosynthesis and stomatal conductance of mature eastern white pine *(Pinus strobus).* Can. J. For. Res. 22: 1387-1394.

Martin, C.A., H.G. Ponder, and C.H. Gilliam. 1989. Effects of irrigation rate and media on growth of *Acer rubrum* L. in containers. Journal of Environmental Horticulture 7(1): 38-40.

Medrano, H., J.M. Escalona, J. Bota, J. Gulias, and J. Flexas. 2002. Regulation of photosynthesis of C-3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Annals of Botany 89: 895-905.

Ningbo, C., T. Dub, S. Kang, F.Li, X. Hua, M. Wang, and Z. Li. 2009. Relationship between stable carbon isotope discrimination and water use efficiency under regulated deficit irrigation of pear-jujube tree. Agricultural Water Management 96: 1615–1622.

NOAA. 2009. NOAA: Summer temperature below average for U.S. National Oceanic and Atmospheric Association. 10 September 2009. <http://www.noaanews.noaa.gov/stories2009/20090910_summerstats.html>

Olivas-Garcia, J.M., B.M. Cregg, and T.C. Hennessey. 2000. Genotypic variation in carbon isotope discrimination and gas exchange of ponderosa pine seedlings under two levels of water stress. Canadian Journal of Forest Research 30:1581-1590.

Reich, P.B. and T.M. Hinckley. 1989. Influence of pre-dawn water potential and soil-to-leaf hydraulic conductance on maximum daily leaf diffusive conductance in two oak species. Functional Ecology 3:719-726.

Reich, P.B., D.S. Ellsworth, and M.B. Walters. 1998. Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. Functional Ecology 12: 948–958.

Roberts, B.R. and V.M. Schnipke. 1987. The water requirements of five container-grown *Acer* species. Hortscience 22(5):1161-1161.

Roussel, M., E. Dreyer, P. Montpied, G. Le-Provost, J.M. Guehl, and O. Brendel. 2009. The diversity of ^{13}C isotope discrimination in a *Quercus robur* full-sib family is associated with differences in intrinsic water use efficiency, transpiration efficiency, and stomatal conductance. Journal of Experimental Botany 60(8):2419-2431.

Ruter, J.M. 1998. Pot-In-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. Journal of Environmental Horticulture 16(3): 159-162.

Taiz, L. and E. Zeiger. Plant Physiology, 4th edn. Sunderland, MA: Sinauer Associates Inc., 2006.

Turnbull, M.H., D. Whitehead, D.T. Tissue, W.S.F Schuster, K.J. Brown, V.C. Engel, and K.L. Griffin. 2002. Photosynthetic characteristics in canopies of *Quercus rubra, Quercus prinus* and *Acer rubrum* differ in response to soil water availability. Oecologia 130:515–524.

Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996a. Cyclic irrigation increases irrigation application efficiency and decreases ammonium losses. Journal of Environmental Horticulture 14(4): 194-198.

Tyler, H. H., S. L. Warren, and T.E. Bilderback. 1996b. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. Journal of Environmental Horticulture 14(4): 199-204.

Warren, S.L. and T.E. Bilderback. 2002. Timing of low pressure irrigation affects plant growth and water utilization efficiency. Journal of Environmental Horticulture 20(3): 184-188.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009a. Water conservation, growth, and water use efficiency of container-grown woody ornamentals irrigated based on daily water use. Hortscience 44(5): 1308-1318.

Warsaw, A.L., R.T. Fernandez, B.M. Cregg, and J.A. Andresen. 2009b. Container-grown ornamental plant growth and water runoff nutrient content and volume under four irrigation treatments. Hortscience 44(6): 1573-1580.

Witmer, R. 2000. Water use of landscape trees during pot-in-pot production and during establishment in the landscape. Horticulture. Blacksburg, Virginia Polytechnic Institute and State University. Doctor of Philosophy: 195.

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

Yoo, C.Y., H.E. Pence, P.M. Hasegawa, and M.V. Mickelbart. 2009. Regulation of transpiration to improve crop water use. Critical Reviews in Plant Sciences 28: (6)410-431.

Zhang, J. and B.M Cregg. 2005. Growth and physiological responses to varied environments among populations of *Pinus ponderosa*. Forest Ecology and Management 219:1-12.

CHAPTER FOUR

SUMMARY AND CONCLUSIONS

Significance of the study

Previous research testing the effects of cyclic irrigation has been conducted mainly in the southeastern U.S. or used smaller plant material (Beeson, 1992; Beeson and Haydu, 1995; Fain et al., 1999; Fare et al., 1996; Ismail et al., 2007; Keever and Cobb, 1985; Ruter, 1998; Tyler et al., 1996). This study was designed to test the effects of cyclic irrigation on the growth of PIP-grown coniferous and deciduous shade trees in the upper Midwest. In addition to classifying the growth response, we explored the physiological mechanisms controlling these growth responses. Carbon isotope discrimination is a parameter often tested in ecological water stress research. Few horticultural studies have used this technique to classify the water use efficiency of trees, as we have. Our results will contribute to the understanding of water stress physiology of container-grown landscape and coniferous trees and aid in the development of water-conserving irrigation guidelines for growers in the upper Midwest.

Main results and ramifications

Deciduous Shade Trees

Seven taxa (*Acer freemanii* 'Jeffersred', *A. rubrum* 'Franksred', *Gleditsia triacanthos* 'Harve', *Gleditsia triacanthos* 'Skyline', *Quercus rubra*, *Ulmus* 'Morton', and *Ulmus* 'Morton Glossy') of bare-root liners were grown in #25 containers and randomly assigned to varying irrigation regimes. Irrigation treatments consisted of varying combinations of irrigation rates (low and high) and daily cycle frequencies (one or four). Low and high irrigation rates corresponded to 1 cm and 2 cm depths, respectively. Applications of the low irrigation rate, regardless of the number of cycles in which it was applied, reduced stem radial growth of all taxa except *A. freemanii* and *G. triacanthos* 'Harve' compared to the high irrigation rate.

Physiological parameters were more responsive to irrigation rate than cycle frequency. Mid-day leaf stomatal conductance (g_S) of trees irrigated cyclically was equal to or less than trees irrigated once daily. Increasing cycle frequency only increased Δ of foliar and wood tissue in *A. rubrum* trees. Integrated water use efficiency (WUE) using carbon isotope analysis of plant tissue is a more sensitive indicator of plant water stress than instantaneous WUE (WUE_i) derived from gas exchange measurements.

Conifers

Picea glauca var. *densata*, *Picea pungens*, *Abies fraseri*, and *Pinus strobus* transplants (plug+2 or 2+2) were planted in #3 containers in a 80:20 (volume:volume) mix of pine bark and peat moss. Also, trees of the same species grown in #3 containers from a previous study were transplanted into #7 containers using the same media. Trees were randomly assigned to irrigation regimes with varying combinations of irrigation rates (low, medium, or high) and daily cycle frequencies (one or four). Low, medium, and high irrigation rates of trees grown in #3 containers corresponded to 1 cm, 2 cm, and 3 cm depths, respectively. Low and high irrigation rates of trees grown in #7 containers corresponded to 1 cm and 2 cm depths, respectively.

Although stem caliper is the best single indicator of tree growth (Thompson, 1985), tree height is the parameter by which coniferous trees are most often sold in the upper Midwest. We suggest growers integrate both measurements and use height: stem caliper as an indicator of plant marketability. Moreover, stem caliper growth is a more sensitive indicator of tree water stress than is tree height. Height growth of only *P. strobus* trees increased under cyclic irrigation regimes, and irrigation rates did not affect height growth. Stem radial growth of only *P. pungens* trees were affected by irrigation rate; the low rate reduced stem radial growth compared to the high rate in both experiments.

Physiological parameters were more responsive to irrigation rate than cycle frequency. Mid-day gas exchange and instantaneous water use efficiency (WUE_i) were unaffected by irrigation treatment. Δ in foliar and wood tissue increased at reduced irrigation rates. Increasing cycle frequency increased Δ in foliar and wood tissue. Medium irrigation rates leached more NO3-N from containers and reduced foliar N content of *P. strobus* and *P. pungens* trees grown in #7 containers compared to the low rate. Trees of equal size can be produced with 50% less water than the typical irrigation rate of 2 cm, thereby increasing profitability for growers by reducing input costs.

The effect of irrigation regimes on WUE_i was highly dependent on environmental conditions. The parameter's inconsistent response demonstrates the method's limitations as an accurate predictor of WUE. Within the #3 containers, the species with the highest WUE_i (*P. strobus*) was most capable of handling water stress and therefore did not experience large reductions in growth as a result of water limiting irrigation regimes. We were unable to link the growth decline of *P. strobus* trees in #7 containers as a result of irrigation cycle frequency to reductions in mid-day gas exchange.

Reducing irrigation rates by 50% is an effective method by which growers in the upper Midwest can conserve water and reduce input costs without sacrificing growth. Reduced foliar N levels and reductions in leachate NO3-N concentration at the 2 cm application rate indicate that growers may leach nutrients and sacrificing nutrient efficacy using these common irrigation methods. However, the decreases in stem radial growth of *P. pungens* at reduced irrigation rates suggests that in some cases growers must choose between increased nutrient retention and growth optimization.

Future research

Our results indicate that cyclic irrigation does not increase tree growth rates of coniferous and deciduous shade trees in the upper Midwest compared to typical once-daily applications. However, cycle frequencies other than the four times per day we tested may be effective in reducing water stress and increasing tree growth rate. The time at which irrigation subvolumes are applied should also be tested, as should various cycle frequency x rate x timing combinations. Testing cyclic irrigation on a broader scale would be helpful in assessing environmental conditions under which tree growth would benefit from increasing cycle frequency. Furthermore, determining the common characteristics among taxa which benefit from cyclic irrigation and reduced irrigation rates will allow growers to group trees based on functional characteristics and irrigate accordingly.

Reductions in mid-day canopy temperatures associated with cyclic irrigation have been reported in past studies (Keever and Cobb, 1985; Graves et al., 1989). This easily measurable parameter should be included in future studies testing cyclic irrigation. Carbon isotope discrimination, regardless of the tissue (wood or leaf) from which it is derived, is a highly sensitive indicator of plant water stress and should be applied more often in horticultural studies. Carbon isotope discrimination is integrative and more reliable than WUE_i measurements derived from gas exchange measurements. Because carbon isotope analysis of tissues can be expensive (≈\$6.50/sample), it is important to consider the period of time over which is of interest. In studies testing the effect of water stress over the entire growing season, it would likely be most cost-effective to select wood tissue for carbon isotope analysis.

To fully assess the reduction in NO3-N by cyclic irrigation regimes, more extensive collections systems must be developed. Leachate collection systems which measure total leachate volume and leachate NO3-N concentration will more accurately quantify NO3-N leaching from container nurseries in the upper Midwest. This will aid in quantifying the effectiveness of water-conserving irrigation programs in reducing the environmental impact.

LITERATURE CITED

Beeson, Jr., R.C. 1992. Restricting overhead irrigation to dawn limits growth in container-grown woody ornamentals. Hortscience 27(9): 996-999.

Beeson, Jr., R. C. and J. Haydu. 1995. Cyclic microirrigation in container-grown landscape plants improves plant growth and water conservation. Journal of Environmental Horticulture 13(1): 6-11.

Fain, G. B., K.M. Tilt, C.H. Gilliam, H.G. Ponder, and J.L. Sibley. 1999. Cyclic irrigation improves irrigation application efficiency and growth of sawtooth oak. Journal of Arboriculture 25(4): 200-203.

Fare, D. C., C.H. Gilliam, G.J. Keever, and R.B. Reed. 1996. Cyclic irrigation and media affect container leachate and ageratum growth. Journal of Environmental Horticulture 14(1): 17-21.

Graves, W. R., M. N. Dana, and R.J. Joly. 1989. Root-zone temperature affects water status and growth of red maple. Journal of the American Society for Horticultural Science 114(3): 406-410.

Ismail, S.M., K. Ozawa, and N.A. Khondaker. 2007. Influence of single and multiple water application timings on yield and water use efficiency in tomato (var. First Power). Agricultural Water Management 95(2008): 116-122.

Keever, G. J. and G. S. Cobb. 1985. Irrigation scheduling effects on container media and canopy temperatures and growth of 'Hershey Red' azalea. Hortscience 20(5): 921-923.

Ruter, J.M. 1998. Pot-In-Pot production and cyclic irrigation influence growth and irrigation efficiency of 'Okame' cherries. Journal of Environmental Horticulture 16(3): 159-162.

Thompson, B.E. 1985. Proceedings: Evaluating seedling quality: principles, procedures, and predictive abilities of major tests workshop. October 16-18, 1984. Forest Research Laboratory, Oregon State University, Corvallis, OR.

Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Cyclic irrigation increases irrigation application efficiency and decreases ammonium losses. Journal of Environmental Horticulture 14(4): 194-198.

APPENDIX 1

Table A1.1. Pearson's correlation values for total height growth, total caliper growth, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), specific leaf area (SLA), total leaf area (TLA), foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *Acer freemanii* trees grown in #25 containers in a PIP production.

	Caliper	A_{max}	g_s	WUE_i	SLA	TLA	CC	Foliar N	Δ wood year one	Δ foliage	Δ wood year two
Height	0.62***	0.11	0.07	-0.01	0.29**	0.69***	0.00	-0.17	0.26**	0.44***	0.05
Caliper		0.21*	0.19**	-0.05	0.06	0.70***	0.03	-0.13	0.55***	0.83***	0.44***
A_{max}			0.95***	-0.11	0.22**	0.23**	0.46***	-0.19*	0.17*	0.21**	0.11
g_s				-0.28**	0.21**	0.25**	0.50***	-0.22**	0.16	0.21**	0.17*
WUE_i					-0.07	-0.09	-0.21**	0.06	0.07	-0.09	-0.02
SLA						0.63***	0.02	-0.40	-0.08	0.15	0.18*
TLA							0.02	-0.49***	0.36***	0.71***	0.54***
CC								0.07	-0.04	0.06	-0.02
Foliar N									-0.40***	-0.08	-0.18*
Δ wood year one										0.25*	0.17*
Δ foliage											0.19*

Table A1.2. Pearson's correlation values for total height growth, total caliper growth, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), specific leaf area (SLA), total leaf area (TLA), foliar chlorophyll content (CC), foliar N concentrations, and $\Delta^{13}C$ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *Acer rubrum* trees grown in #25 containers in a PIP production.

	Caliper	A_{max}	g_s	WUE_i	SLA	TLA	CC	Foliar N	Δ wood year one	Δ foliage	Δ wood year two
Height	0.71***	0.07	0.07	-0.08	0.54***	0.64***	0.03	-0.32***	-0.10	0.29**	0.52***
Caliper		0.04	0.00	-0.05	0.47***	0.90***	-0.03	-0.69***	-0.12	0.55***	0.31***
A_{max}			0.91***	-0.37***	0.11	0.04	0.12	0.04	0.04	-0.04	0.12
g_s				-0.60***	0.03	-0.02	0.20**	0.08	0.03	-0.04	0.11
WUE_i					-0.07	-0.03	-0.23**	0.06	0.00	-0.06	-0.14
SLA						0.67***	0.02	-0.41***	0.19*	0.21**	0.70***
TLA							-0.04	-0.76***	0.09	0.49***	0.40***
CC								0.09	0.00	-0.07	0.00
Foliar N									0.17*	-0.69***	-0.30***
Δ wood year one										0.00	0.33***
Δ foliage											0.12

Table A1.3. Pearson's correlation values for total height growth, total caliper growth, foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *G. triacanthos* 'Harve' trees grown in #25 containers in a PIP production.

	Caliper	CC	Foliar N	Δ wood year one	Δ wood year two
Height	0.47***	0.02	-0.44***	-0.38***	0.00
Caliper		0.10	-0.47***	-0.61***	0.27
CC			-0.03	0.18	0.13
Foliar N				0.49***	-0.14

Table A1.4. Pearson's correlation values for total height growth, total caliper growth, foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *G. triacanthos* 'Skyline' trees grown in #25 containers in a PIP production.

	Caliper	CC	Foliar N	Δ wood year one	Δ wood year two
Height	0.57***	-0.06	-0.19*	-0.12	-0.29**
Caliper		0.01	-0.26**	0.17	-0.30**
CC			0.01	0.03	0.11
Foliar N				0.15	-0.02

Table A1.5. Pearson's correlation values for total height growth, total caliper growth, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), specific leaf area (SLA), total leaf area (TLA), foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *Quercus rubra* trees grown in #25 containers in a PIP production.

	Caliper	A_{max}	g_s	WUE_i	SLA	TLA	CC	Foliar N	Δ wood year one	Δ foliage	Δ wood year two
Height	0.19*	-0.05	0.05	-0.06	0.17*	0.11	-0.02	0.20*	-0.43***	0.28**	0.50***
Caliper		0.26**	0.27**	-0.18*	0.41***	0.90***	-0.03	-0.31***	0.01	0.80***	0.25**
A_{max}			0.92***	-0.12	0.22**	0.29**	0.20*	-0.06	-0.03	0.17*	-0.03
g_s				-0.34***	0.16*	0.24*	0.20*	0.02	-0.10	0.22*	0.05
WUE_i					0.09	-0.09	-0.16*	-0.07	0.06	-0.08	0.06
SLA						0.69***	-0.16*	-0.33***	0.01	0.50***	0.32***
TLA							-0.09	-0.41***	0.05	0.72***	0.22*
CC								0.08	-0.03	-0.07	-0.14
Foliar N									-0.51***	-0.36***	-0.09
Δ wood year one										-0.03	0.01
Δ foliage											0.75***

Table A1.6. Pearson's correlation values for total height growth, total caliper growth, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), specific leaf area (SLA), total leaf area (TLA), foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *Ulmus* 'Morton' trees grown in #25 containers in a PIP production.

	Caliper	A_{max}	g_s	WUE_i	SLA	TLA	CC	Foliar N	Δ wood year one	Δ foliage	Δ wood year two
Height	0.48***	0.12	0.06	0.06	0.32***	0.58***	0.01	-0.04	0.45***	0.19*	0.59***
Caliper		0.16*	0.19*	-0.02	0.05	0.65***	-0.02	-0.55***	0.41***	0.41***	0.24**
A_{max}			0.88***	0.21*	0.17*	0.21*	0.01	-0.01	-0.10	-0.01	-0.05
g_s				-0.16*	0.09	0.16*	0.02	-0.06	0.00	0.12	0.03
WUE_i					0.09	0.05	-0.16*	0.09	-0.12	-0.17*	-0.07
SLA						0.78***	-0.01	-0.06	-0.02	-0.08	0.09
TLA							-0.02	-0.43***	0.29**	0.20*	0.26**
CC								0.10	-0.01	-0.06	0.00
Foliar N									-0.25**	-0.47***	-0.05
Δ wood year one										0.39***	0.75***
Δ foliage											0.59***

Table A1.7. Pearson's correlation values for total height growth, total caliper growth, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), specific leaf area (SLA), total leaf area (TLA), foliar chlorophyll content (CC), foliar N concentrations, and Δ values of wood tissue formed in year one and year two of the study and foliar tissue from the second year of the study of *Ulmus* 'Morton Glossy' trees grown in #25 containers in a PIP production.

	Caliper	A_{max}	g_s	WUE_i	SLA	TLA	CC	Foliar N	Δ wood year one	Δ foliage	Δ wood year two
Height	0.19**	-0.02	-0.07	0.14	0.21**	0.43***	0.03	-0.29***	0.15	0.28**	-0.43***
Caliper		0.02	-0.02	0.00	0.03	0.62***	0.00	-0.33***	0.05	0.34***	-0.05
A_{max}			0.92***	-0.51***	0.20**	0.14	0.06	0.12	0.10	0.23**	-0.14
g_s				-0.69***	0.24***	0.13	0.14	0.24**	0.11	0.27**	-0.14
WUE_i					-0.14	-0.06	-0.10	-0.27**	-0.05	-0.18*	0.07
SLA						0.75***	0.02	0.22**	-0.02	0.33***	-0.38***
TLA							0.01	-0.16*	0.16*	0.45***	-0.37***
CC								0.11	-0.02	-0.02	-0.08
Foliar N									-0.06	-0.01	-0.15
Δ wood year one										0.36***	0.52***
Δ foliage											-0.07

Table A1.8: F-values from ANOVA for mid-day WUE$_i$ (A_{max}/g_S) of five taxa of deciduous shade trees grown in #25 containers under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) (α=0.05).

	Source of Variation	d.f.	2008		2009				
			25 June	28 July	5 June	9 July	14 May	31 July	4 Sept
A. freemanii	Cycles	1	0.68	4.57	0.44	3.01	1.12	0.41	8.29
	Rate	1	0.58	0.00	3.90	1.94	0.39	2.32	0.77
	Cycles*Rate	1	0.91	0.19	1.55	1.15	1.29	5.68	1.88
A. rubrum	Cycles	1	2.62	0.52	5.44	1.33	0.66	13.82*	1.66
	Rate	1	0.50	1.68	0.35	1.71	0.02	1.16	0.02
	Cycles*Rate	1	0.64	2.95	27.98**	0.00	0.51	0.52	0.16
Q. rubra	Cycles	1	1.01	2.05	0.62	0.99	1.09	0.00	4.34
	Rate	1	3.74	3.85	0.71	0.92	1.48	0.24	5.38
	Cycles*Rate	1	2.15	3.91	0.91	0.11	0.14	0.43	0.97
Ulmus 'Morton'	Cycles	1	0.00	1.29	0.21	1.20	2.72	0.03	0.06
	Rate	1	1.92	0.91	0.15	2.37	0.19	4.12	0.52
	Cycles*Rate	1	0.09	0.07	0.93	0.80	0.04	4.38	0.04
Ulmus 'Morton Glossy'	Cycles	1	0.15	3.15	0.95	2.90	1.98	2.01	11.49*
	Rate	1	1.37	4.65	3.09	2.69	0.02	0.01	1.00
	Cycles*Rate	1	1.79	7.87	0.79	3.70	0.00	0.33	1.35

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

Figure A1.1: Diurnal course of substrate volumetric moisture content (VWC; %) of a subsample of 16 deciduous shade trees grown in #25 containers in a PIP facility under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) (n=4).

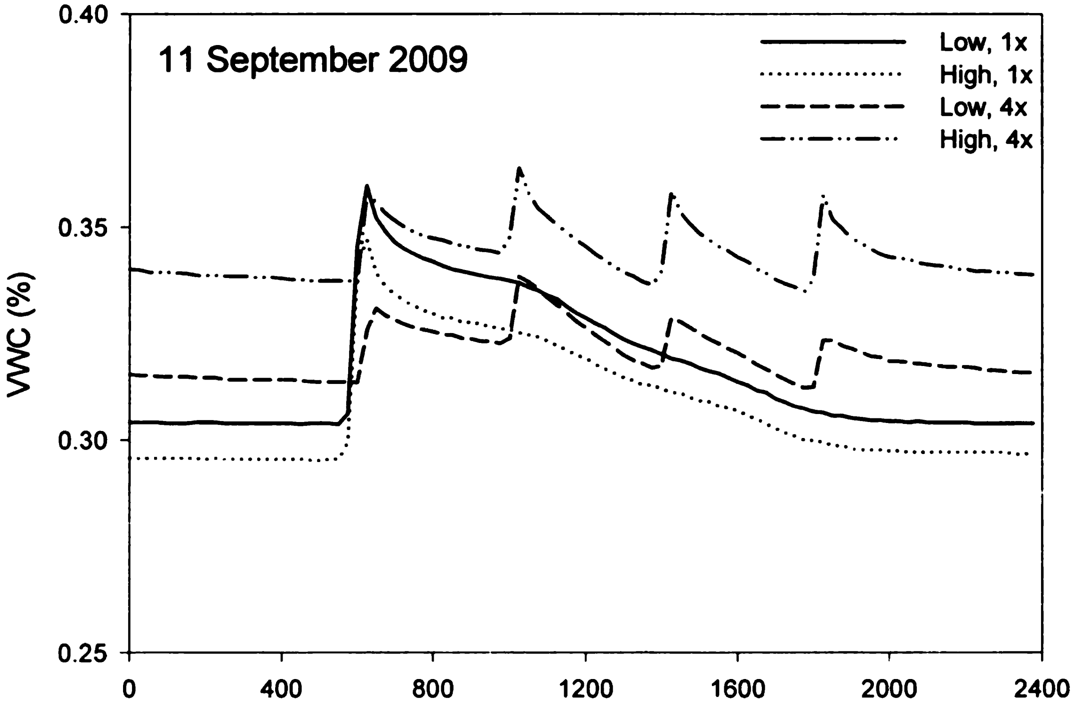


Figure A1.2: Mean NO3-N concentration (ppm), EC (dS/M), and pH (±SE) of pourthru leachate samples collected in 2008 and 2009 from *Acer freemanii*, *Ulmus* 'Morton Glossy', and *G. triacanthos* 'Skyline' trees grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four) on four dates. Effect of species was not significant. Effect of cycle frequency was most often not significant. Data averaged across species and cycle frequencies (n=24). When there was a significant cycles x rate interaction, means were separated by treatments (n=12; * $p \leq 0.05$; ** $p \leq 0.01$).

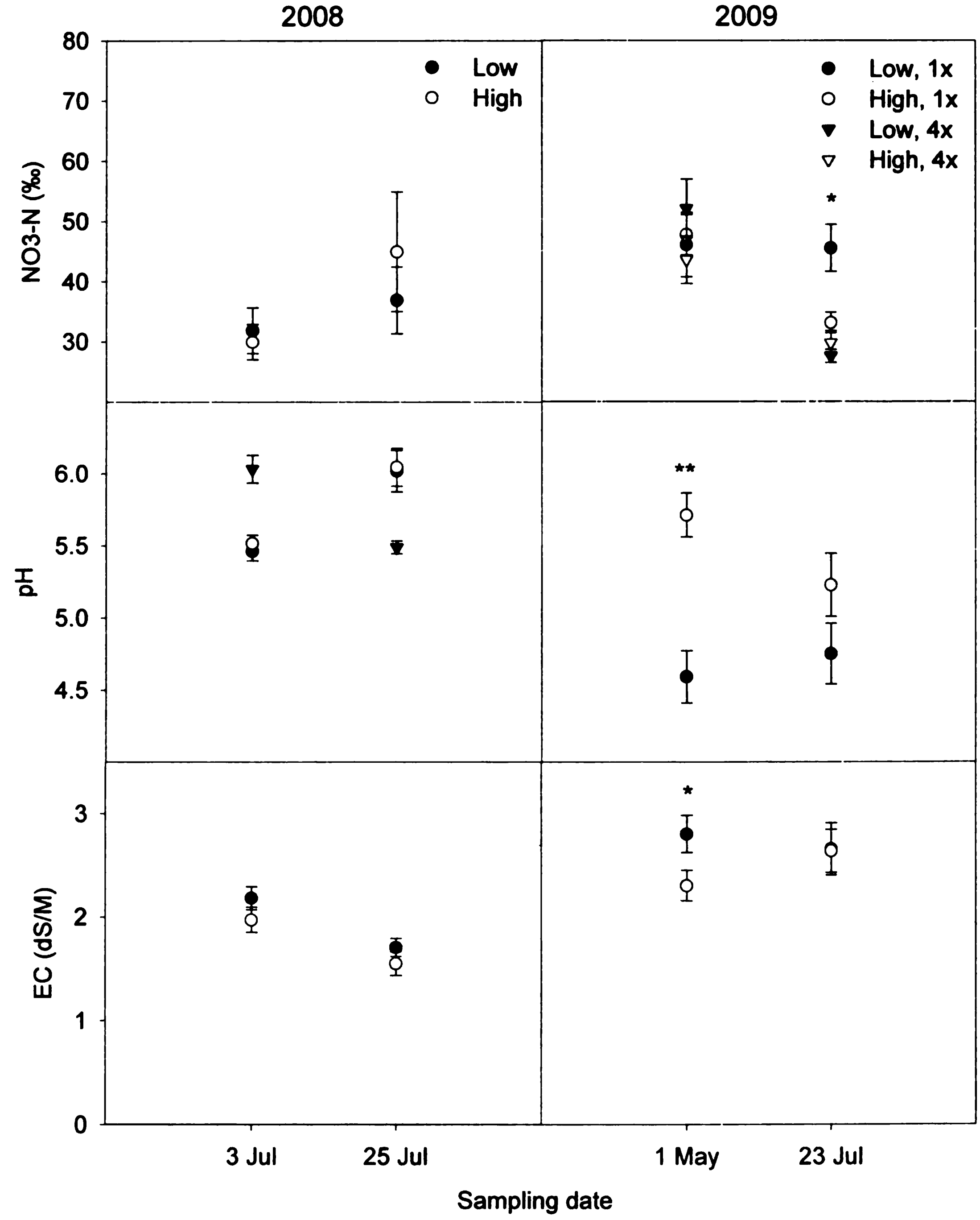


Figure A1.3: Mean height growth (±SE) over the two-year production cycle of seven taxa of deciduous trees grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four4) (n=4; * $p \leq 0.05$). Effect of cycle frequency was not significant.

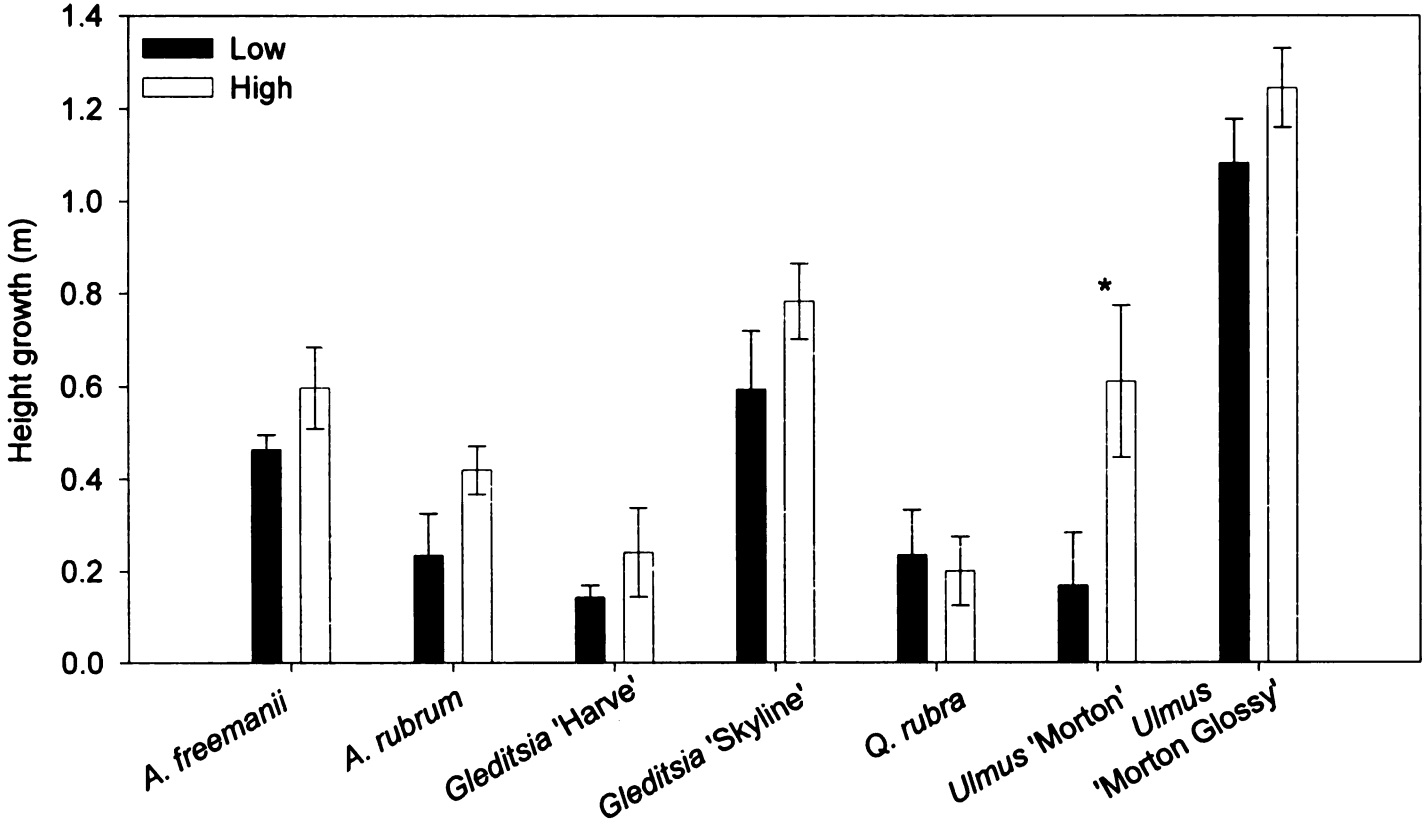
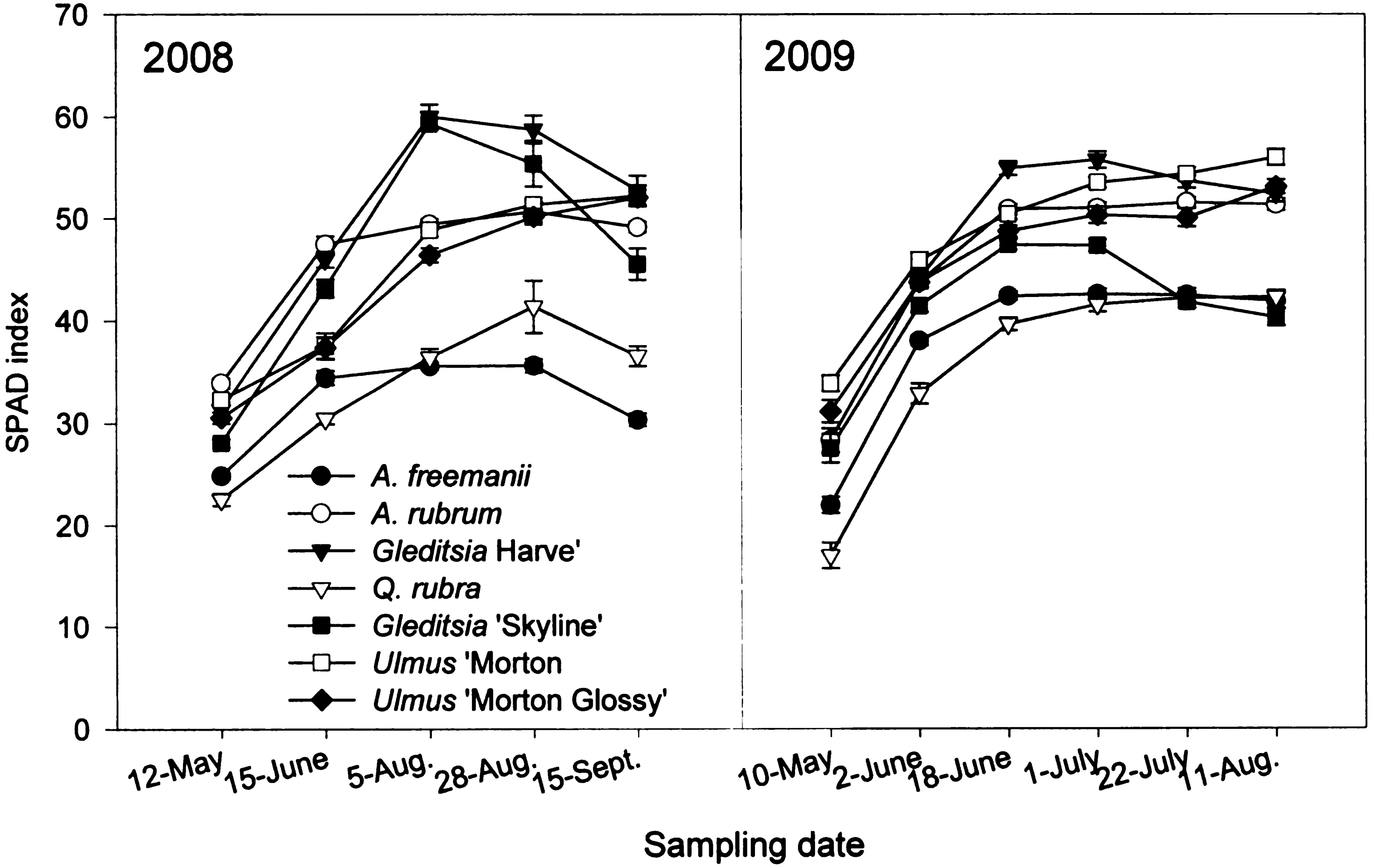


Figure A1.4: Annual courses of SPAD index values of seven deciduous shade tree species grown in #25 containers in a PIP production system under varying combinations of irrigation rate (low, 1 cm or high, 2 cm) and daily cycle frequencies (one or four). Data averaged across treatments (n=16).



APPENDIX 2

Table A2.2. Pearson's correlation values for total caliper growth, total height growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_S), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_S), and Δ values of wood and foliar tissue formed in 2008 and 2009 of *P. glauca* trees grown in #3 containers in a PIP production system.

	Height	Biomass	A_{max}	g_S	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.42***	0.68***	-0.14	-0.05	-0.11	0.44***	-0.33*	0.00	-0.39**	-0.50***
Height		0.65***	-0.12	-0.03	0.06	0.36***	-0.11	-0.09	-0.03	-0.38**
Biomass			-0.18	-0.06	-0.24*	0.46***	-0.23	0.35*	-0.05	-0.25
A_{max}				0.48***	0.05	-0.09	0.15	-0.12	0.10	0.14
g_S					-0.03	-0.14	0.39*	0.00	0.43**	0.23
Foliar N						0.19*	-0.33*	-0.60**	-0.48**	-0.46**
WUE_i							-0.59***	-0.37**	-0.44**	-0.59***
Δ 2008 foliage								0.18	0.71***	0.59***
Δ 2008 wood									0.37**	0.58***
Δ 2009 foliage										0.79***

Table A2.3. Pearson's correlation values for total caliper growth, total height growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ values of wood and foliar tissue formed in 2008 and 2009 of *P. pungens* trees grown in #3 containers in a PIP production system.

	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.49***	0.76***	0.02	0.12	-0.37***	0.25**	0.23	-0.04	0.29*	0.39*
Height		0.59***	-0.04	-0.02	-0.35**	0.48***	0.30*	-0.08	0.47**	0.48**
Biomass			-0.02	0.05	-0.54***	0.41***	0.12	-0.04	0.26	0.28*
A_{max}				0.54***	0.01	-0.09	0.32	-0.15	0.13	0.00
g_s					-0.08	-0.01	0.33	-0.28	0.28	0.14
Foliar N						0.11	-0.46**	0.12	-0.57**	-0.67***
WUE_i							-0.25	-0.16	-0.10	-0.23
Δ 2008 foliage								0.16	0.72***	0.70***
Δ 2008 wood									0.06	0.09
Δ 2009 foliage										0.88***

Table A2.4. Pearson's correlation values for total caliper growth, total height growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ of wood and foliar tissue formed in 2008 and 2009 of *A. fraseri* trees grown in #3 containers in a PIP production system.

	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.04	0.78***	0.04	0.17	0.21*	0.40***	-0.11	-0.23	-0.28	-0.02
Height		0.59***	-0.17	-0.28**	0.15	0.34***	0.04	-0.08	-0.25	-0.28*
Biomass			-0.18	-0.18	0.12	0.54***	-0.20	-0.23	-0.49**	-0.42***
A_{max}				0.52***	0.11	-0.23*	0.15	0.20	0.20	0.15
g_s					-0.04	-0.24*	0.21	0.18	0.33	0.34*
Foliar N						0.37***	-0.60**	-0.60***	-0.70***	-0.59**
WUE_i							-0.41**	-0.56***	-0.48**	-0.34*
Δ 2008 foliage								0.82***	0.70***	0.59***
Δ 2008 wood									0.54**	0.48**
Δ 2009 foliage										0.87***

Table A2.5. Pearson's correlation values for total caliper growth, total height growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ of wood tissue formed in 2008 and 2009 and foliar tissue formed in 2009 of *P. strobus* trees grown in #3 containers in a PIP production system.

							Δ	Δ	Δ
	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	2008 wood	2009 foliage	2009 wood
Caliper	0.36***	0.56***	0.14	0.16	-0.43***	0.08	-0.09	-0.08	0.05
Height		0.57***	0.02	0.06	-0.23*	0.35***	-0.15	-0.37*	-0.38*
Biomass			0.08	0.15	-0.61***	0.38***	-0.15	-0.16	-0.25
A_{max}				0.93***	-0.15	-0.24*	0.26	0.27	0.33
g_s					-0.26*	-0.23*	0.31	0.38	0.40*
Foliar N						0.12	-0.59**	-0.85***	-0.72***
WUE_i							-0.57***	-0.69***	-0.80***
Δ 2008 wood								0.69***	0.76***
Δ 2009 foliage									0.91***

Table A2.6. Pearson's correlation values for total caliper growth, total height growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ of wood and foliar tissue formed in 2008 and 2009 of *P. glauca* trees grown in #7 containers in a PIP production system.

	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.39**	0.84***	0.05	0.08	-0.36*	0.02	0.06	0.10	0.24	0.28
Height		0.61***	-0.04	-0.01	0.37**	0.19	-0.31	0.21	-0.18	-0.31*
Biomass			0.01	0.02	-0.21	0.18	-0.16	0.04	-0.03	-0.05
A_{max}				0.91***	-0.11	-0.31*	-0.17	-0.09	0.04	0.06
g_s					-0.12	-0.50**	-0.09	0.03	0.18	0.16
Foliar N						0.01	0.03	0.01	-0.21	-0.54***
WUE_i							-0.24	-0.10	-0.29	-0.16
Δ2008 foliage								0.13	0.54**	0.28
Δ 2008 wood									0.61***	0.61***
Δ 2009 foliage										0.77***

Table A2.7. Pearson's correlation values for total height growth, total caliper growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ of wood and foliar tissue formed in 2008 and 2009 of *P. pungens* trees grown in #7 containers in a PIP production system.

	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.15	0.57***	0.02	0.11	-0.51**	-0.04	0.68***	0.53**	0.65**	0.27
Height		0.67***	-0.19	-0.22	-0.44**	0.31*	-0.39	-0.05	-0.20	-0.50**
Biomass			-0.11	-0.05	-0.55**	0.20	0.00	0.16	0.04	-0.13
A_{max}				0.86***	0.02	-0.34*	0.23	0.31*	0.39*	0.43**
g_s					0.00	-0.58***	0.21	0.29	0.37	0.41**
Foliar N						-0.10	-0.53**	-0.63***	-0.51**	-0.16
WUE_i							0.08	-0.03	-0.06	-0.14
Δ 2008 foliage								0.89***	0.93***	0.82***
Δ 2008 wood									0.95***	0.73***
Δ 2009 foliage										0.81***

Table A2.8. Pearson's correlation values for total height growth, total caliper growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_S), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_S), and Δ of wood and foliar tissue formed in 2008 and 2009 of *A. fraseri* trees grown in #7 containers in a PIP production system.

	Height	Biomass	A_{max}	g_S	Foliar N	WUE_i	Δ 2008 foliage	Δ 2008 wood	Δ 2009 foliage	Δ 2009 wood
Caliper	0.67***	0.92***	-0.09	0.03	-0.54**	-0.22	0.55**	0.24	0.67**	0.57***
Height		0.76***	-0.04	0.11	-0.32*	-0.23	0.45*	0.05	0.63**	0.45**
Biomass			-0.18	-0.06	-0.46**	-0.21	0.56**	0.10	0.78***	0.61***
A_{max}				0.75***	-0.01	0.24	0.08	0.36*	-0.41*	0.06
g_S					0.02	-0.39**	0.18	0.22	-0.36	0.16
Foliar N						-0.02	-0.38*	-0.47**	-0.50**	-0.55***
WUE_i							-0.21	0.18	-0.13	-0.14
Δ 2008 foliage								0.70***	0.76***	0.85***
Δ 2008 wood									0.26	0.57***
Δ 2009 foliage										0.69***

Table A2.9. Pearson's correlation values for total height growth, total caliper growth, total biomass production, mid-day photosynthetic rate (A_{max}) and leaf stomatal conductance (g_s), foliar N concentrations, mid-day instantaneous water use efficiency (WUE_i; A_{max}/g_s), and Δ of wood tissue formed in 2008 and 2009 and foliar tissue formed in 2009 of *P. strobus* trees grown in #7 containers in a PIP production system.

							Δ	Δ	Δ
	Height	Biomass	A_{max}	g_s	Foliar N	WUE_i	2008 wood	2009 foliage	2009 wood
Caliper	0.40*	0.74***	0.37	0.14	-0.36	0.17	0.23	0.39	0.30
Height		0.66***	0.10	-0.04	-0.39*	0.09	0.04	0.20	0.20
Biomass			0.24	0.05	-0.64***	0.11	0.23	0.50*	0.33
A_{max}				-0.06	-0.12	0.70***	0.40*	0.54*	0.53**
g_s					0.06	-0.71***	0.30	0.31	0.30
Foliar N						-0.14	-0.24	-0.54*	-0.31
WUE_i							0.12	0.26	0.16
Δ 2008 wood								0.75***	0.66***
Δ 2009 foliage									0.56**

Figure A2.1: Height growth over the two-year production cycle of four coniferous tree species grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1, 2, or 3 cm) and cycle frequency (one or four) (n=12).

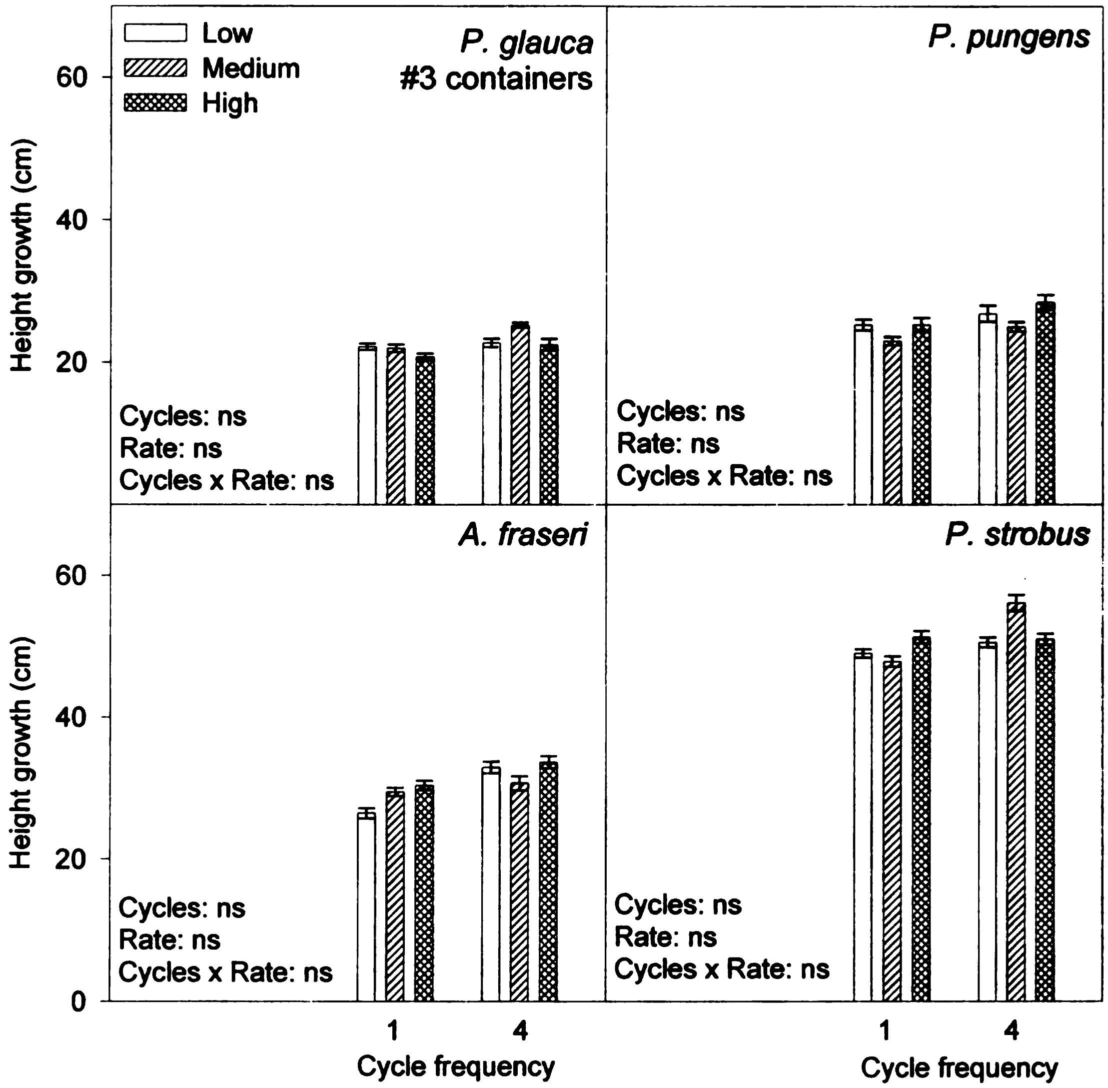


Figure A2.2: Height growth over the two-year production cycle of four coniferous tree species grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1, 2, or 3 cm) and cycle frequency (one or four) (n=4).

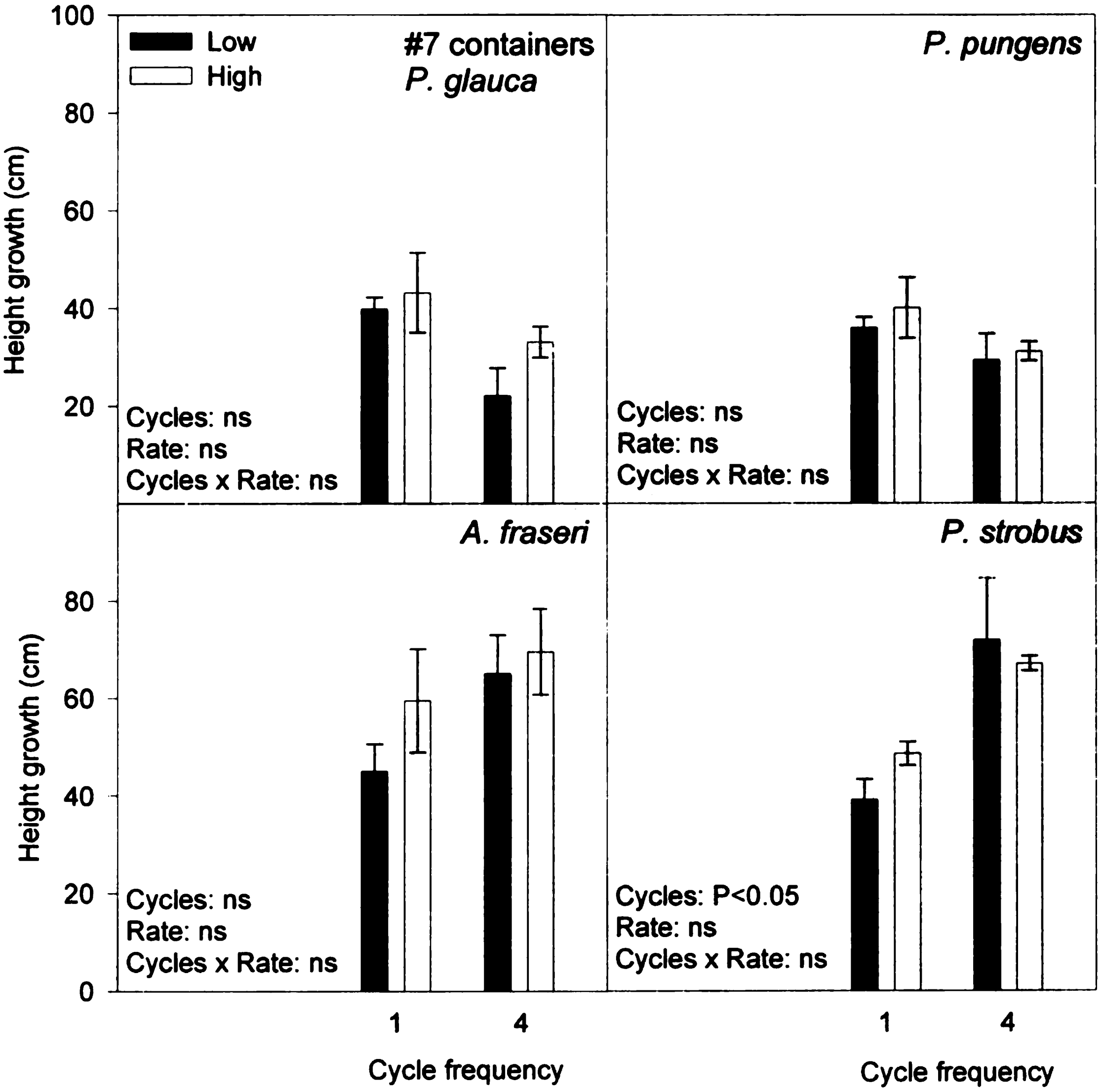


Figure A2.3: Stem radial growth over the two-year production cycle of four coniferous tree species grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1, 2, or 3 cm) and cycle frequency (one or four) (n=12).

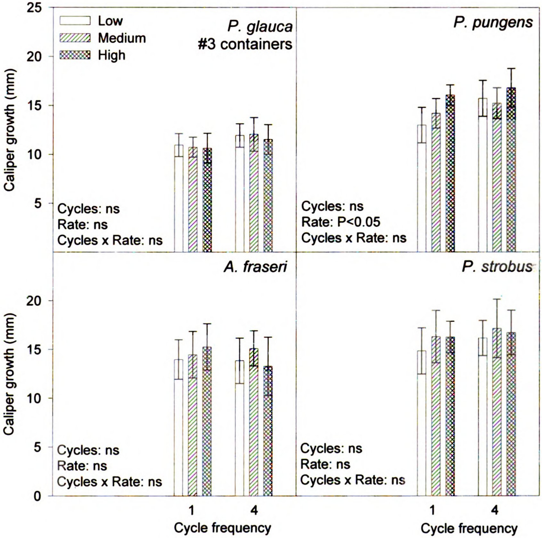
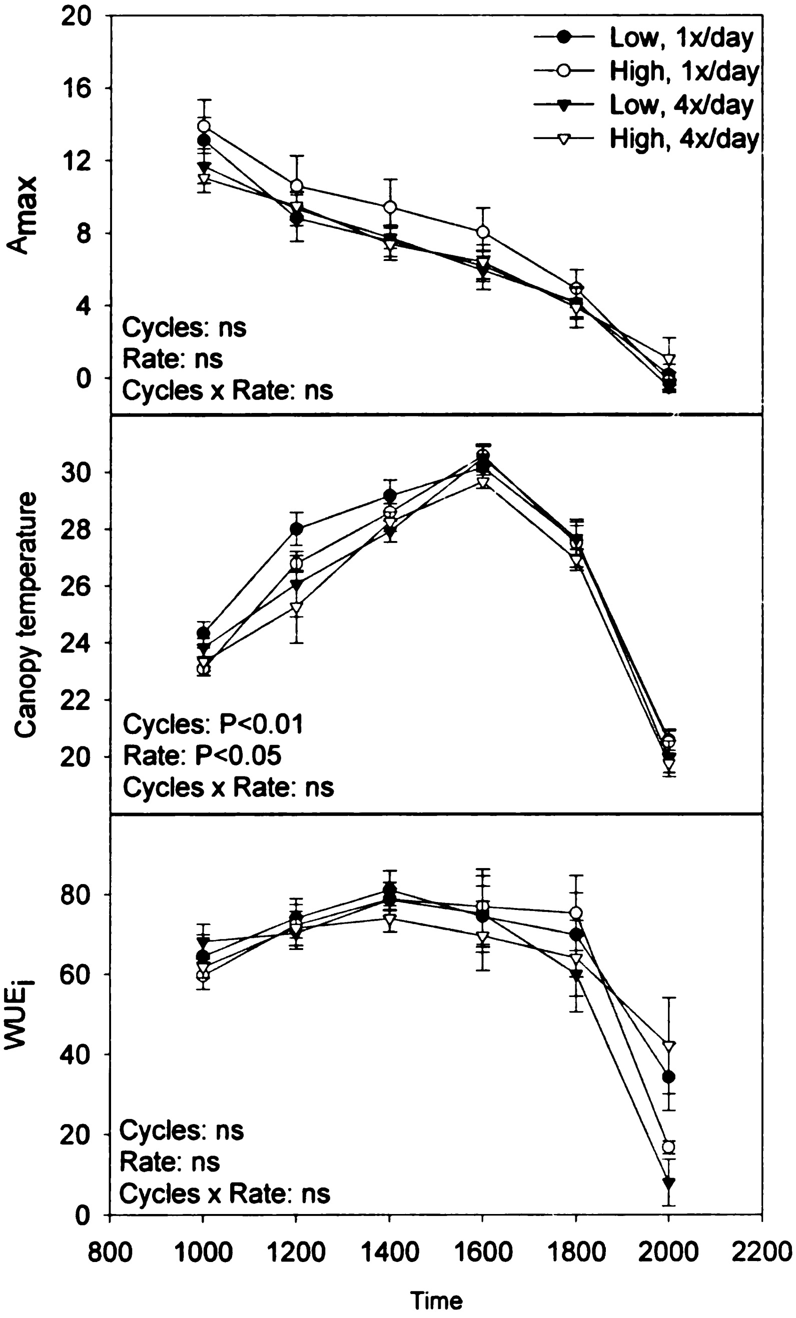


Figure A2.4: Diurnal course of net photosynthesis (A_{max}; μmol•m^{-2}•s^{-1}), canopy temperature (C), and instantaneous water use efficiency (WUE_i; A_{max}/g_s) (±SE) of *A. fraseri* trees grown in #7 containers on 5 August 2009 in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (one or four). Mean diurnal canopy temperature was reduced when trees received the high rate or cyclic irrigation compared to the low rate or once daily applications, respectively (α=0.05; n=4).



s

Figure A2.5: Δ values (±SE) of 2008 and 2009 needles of four coniferous tree species grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1 or 3 cm) and daily cycle frequencies (one or four) (α=0.05; n=6).

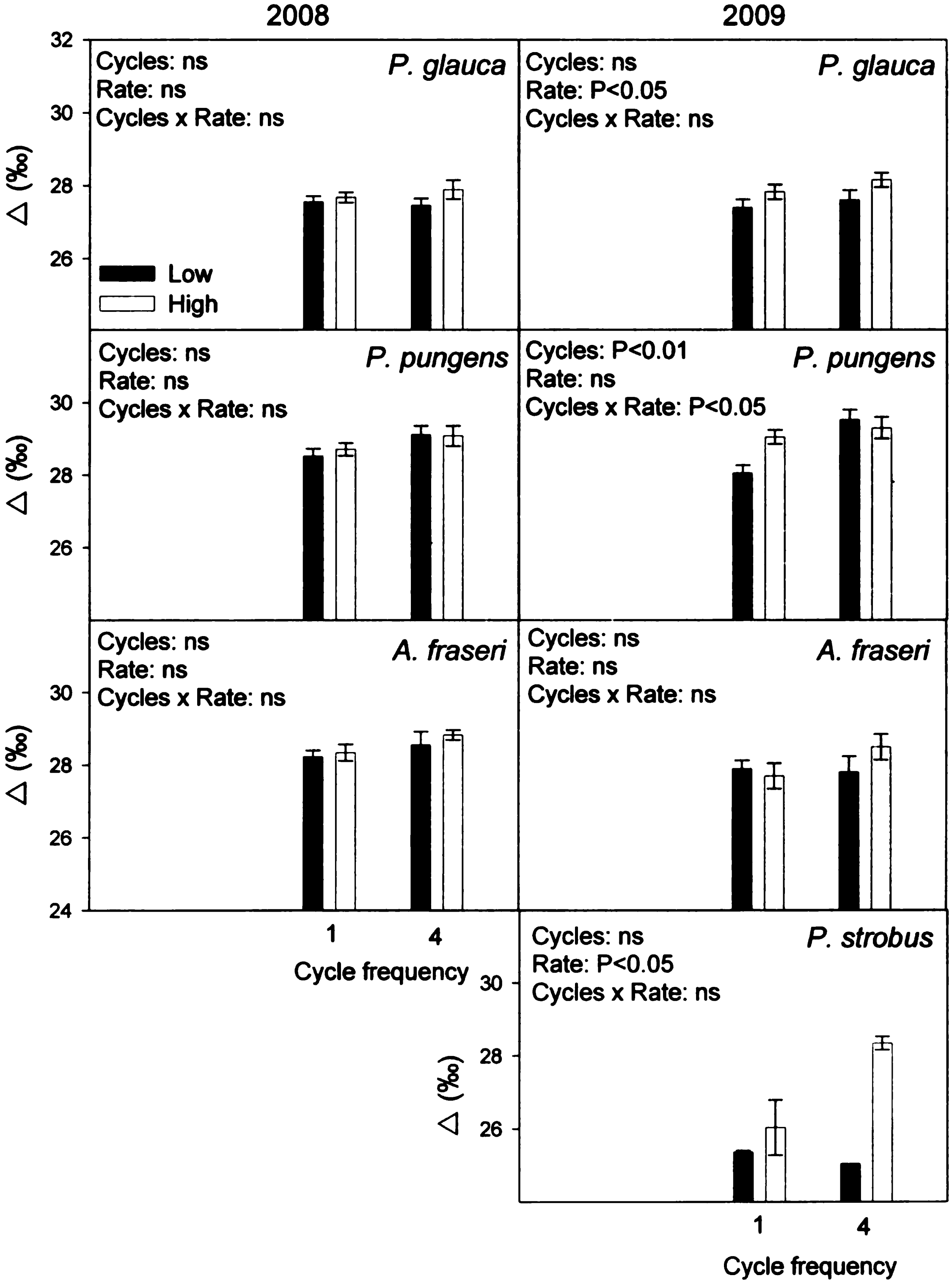


Figure A2.6: Δ values (±SE) of 2008 and 2009 wood of four coniferous tree species grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1 or 3 cm) and daily cycle frequencies (one or four) (α=0.05; n=6).

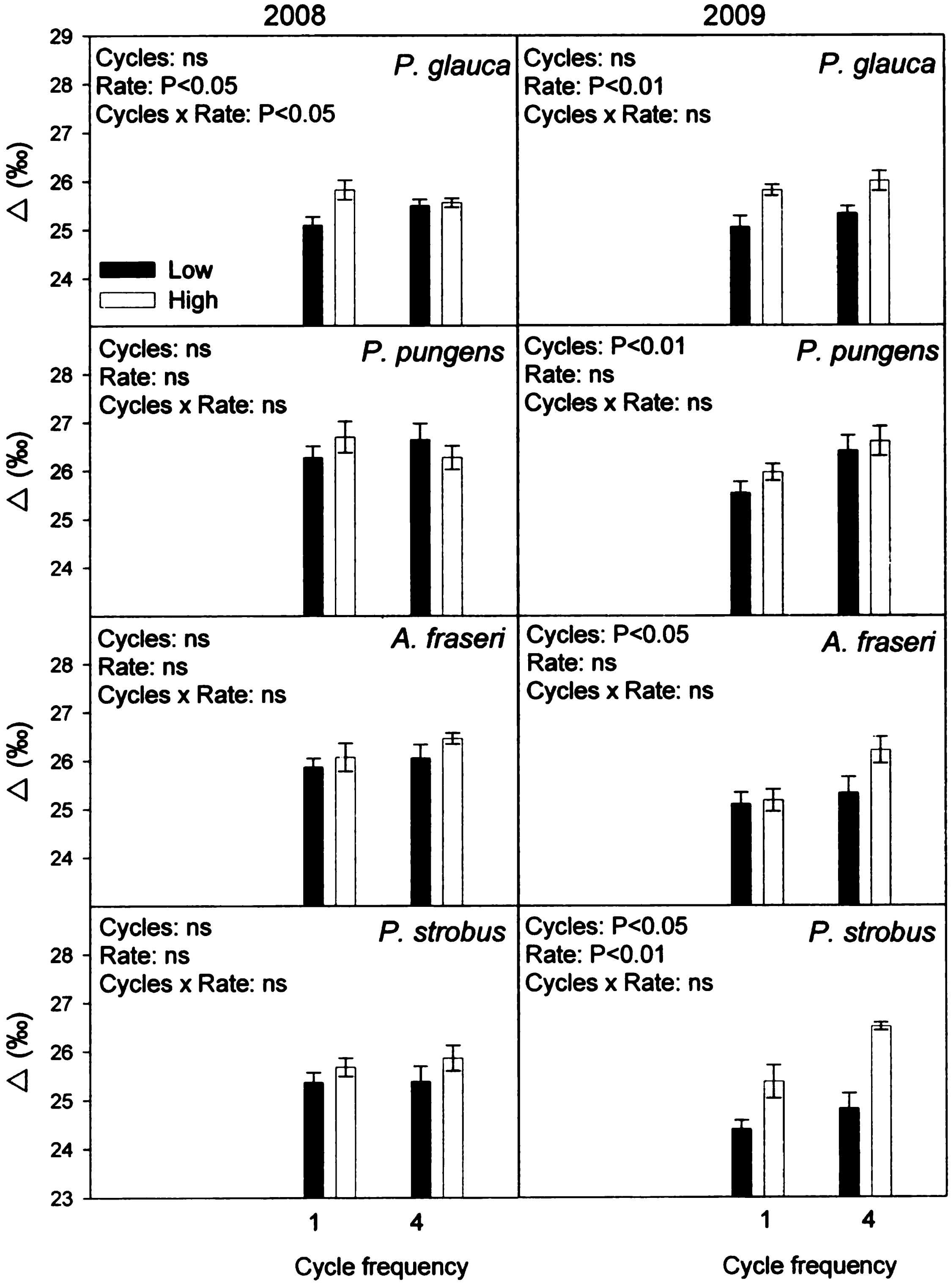


Figure A2.7: Δ values (±SE) of 2008 and 2009 foliar tissue of four coniferous tree species grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (one or four) (α=0.05; n=4).

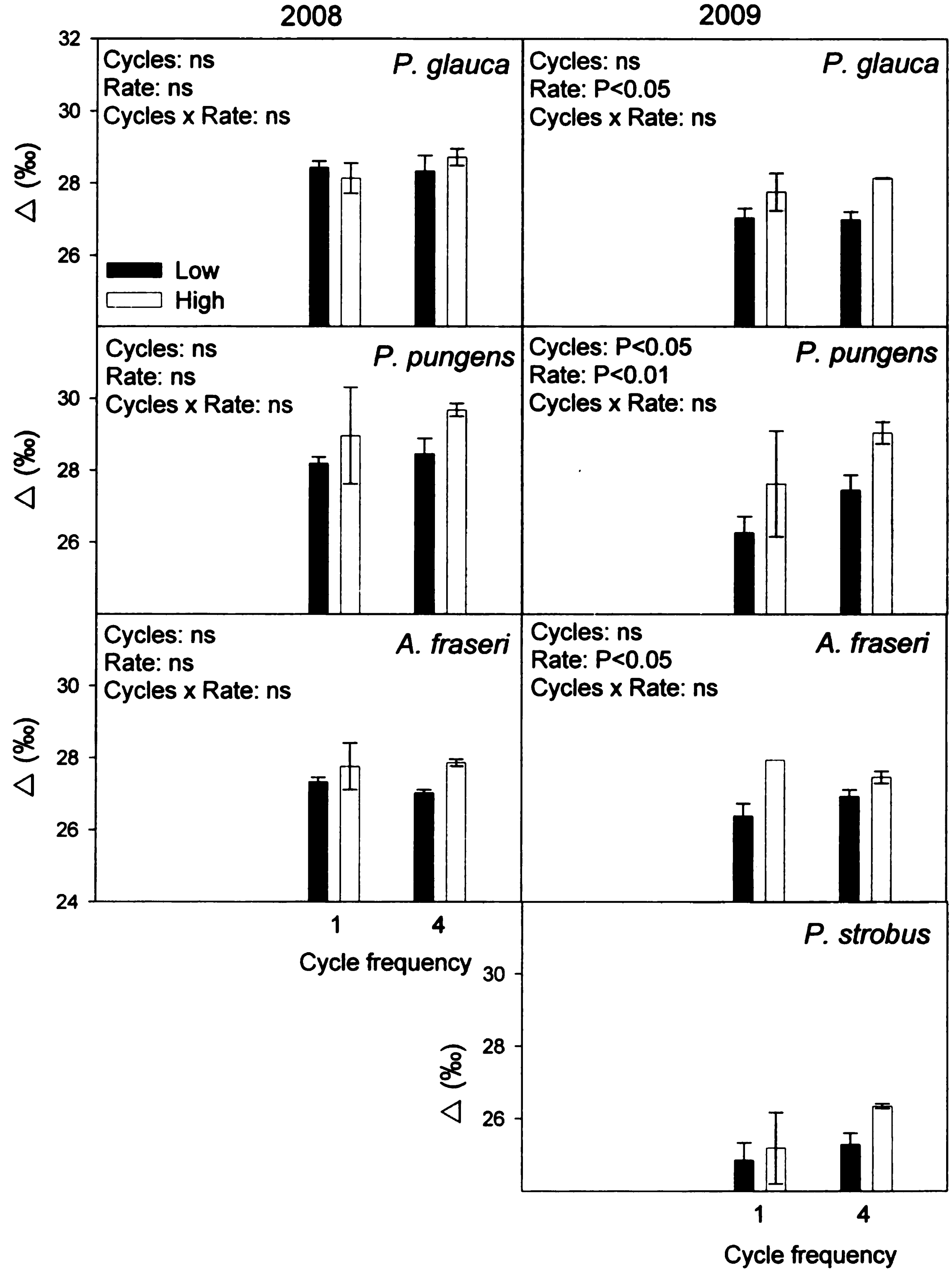


Figure A2.8: Nitrate-N concentration (ppm), EC (dS/M), and pH (±SE) of pourthru leachate samples collected in 2008 and 2009 from *P. strobus* and *A. fraseri* trees grown in #3 containers in a PIP production system under varying combinations of irrigation rate (1, 2, or 3 cm) and daily cycle frequencies (one or four) (n=16). Effect of cycles was not significant; data averaged across species and cycle frequency (n=12). Means with different letters are different at p≤0.05; ^{ns} nonsignificant.

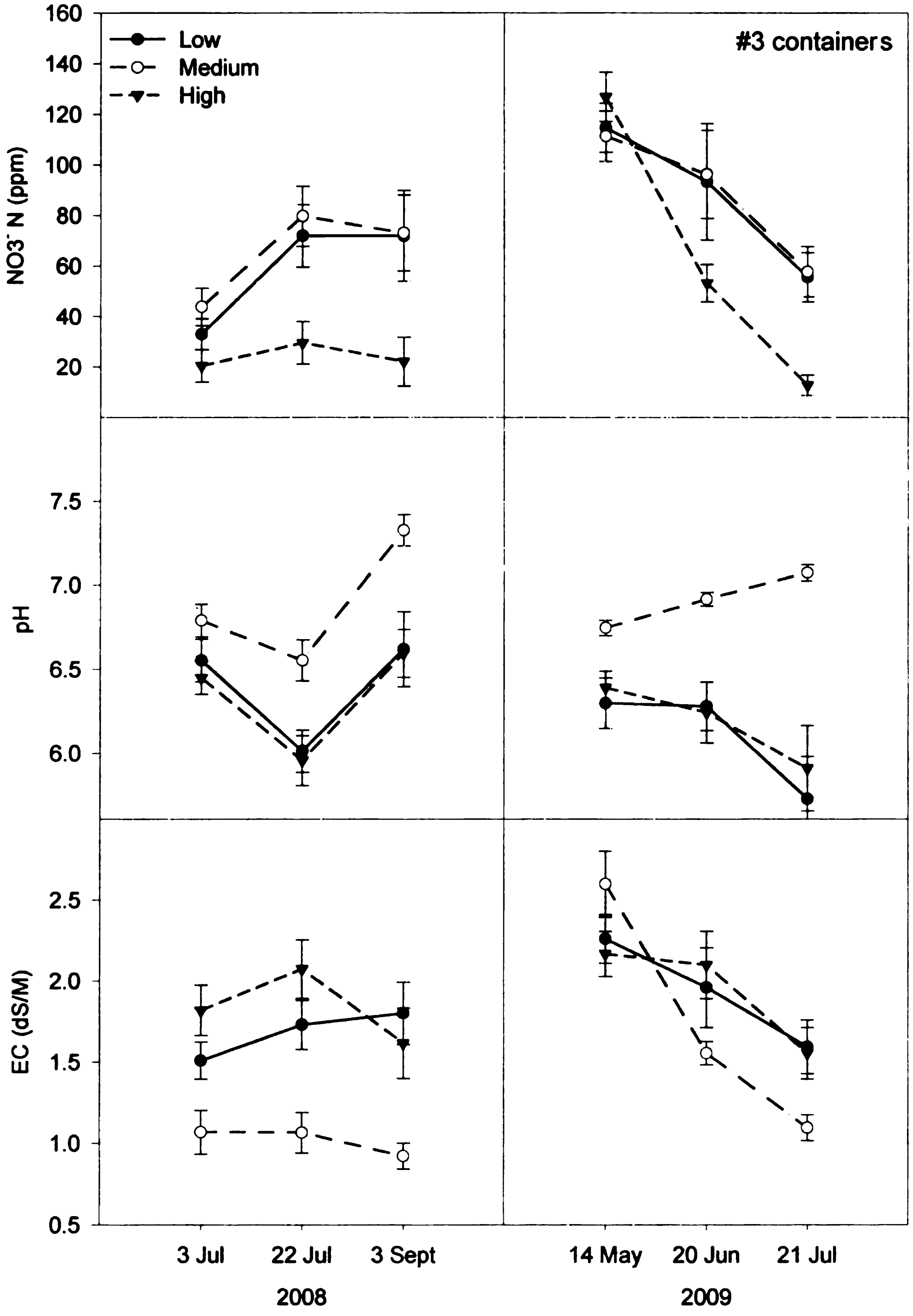


Figure A2.9: NO3-N concentration (ppm), EC (dS/M), and pH (±SE) of pourthru leachate samples collected in 2008 and 2009 from *P. strobus* and *A. fraseri* trees grown in #7 containers in a PIP production system under varying combinations of irrigation rate (1 or 2 cm) and daily cycle frequencies (1 or 4). Effect of cycles was not significant; data averaged across species and cycle frequency (n=12). Means with different letters are different at $p \leq 0.05$; ^{ns} nonsignificant.