THE EFFECT OF IRRIGATION FREQUENCY ON GROWTH AND PHYSIOLOGY OF NATIVE LANDSCAPE SHRUB SPECIES

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A Thesis
Submitted to
the Graduate Faculty of
Auburn University
in Partial Fulfillment of the
Requirements for the
Degree of
Master of Science

Auburn, Alabama
December 17, 2007

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Matthew Fudge Wilkin, son of Edwin and Susan Wilkin, was born in Albany, Georgia on November 5, 1981. He has one sister, Anna. In 2000 he graduated from Miller County High School where he went to Faulkner State Community College for one year before enrolling at The University of Georgia. In the spring of 2005 he graduated with a Bachelor of Science in Agriculture specializing in horticulture. After graduation he enrolled at Auburn University to pursue a Master of Science degree in horticulture. He received his Master’s Monday, December 17, 2007.

THESIS ABSTRACT

THE EFFECT OF IRRIGATION FREQUENCY ON GROWTH AND PHYSIOLOGY OF NATIVE LANDSCAPE SHRUB SPECIES

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Master of Science, December 17, 2007
(Bachelor of Science, Horticulture, The University of Georgia, 2005)

102 typed pages

Directed by Amy N. Wright

Studies were conducted utilizing the Horhizotron™ to evaluate horizontal root growth of *Morella cerifera* (L.) Small (syn. *Myrica cerifera)* (wax myrtle) and *Illicium floridanum* Ellis (Florida anise tree) when portions of the root system were exposed to different levels of soil moisture. Four different irrigation frequencies were randomly assigned among the four quadrants in each Horhizotron and consisted of: watered daily, unwatered, and watered once 10% or 15% moisture by volume was reached. In the first experiment, *M. cerifera* and *I. floridanum* HRL decreased with decreasing irrigation frequency as did root dry weights. In the second experiment no difference in HRL or root dry weights was seen among treatments for *M. cerifera* however, HRL and root dry weight decreased with decreasing irrigation frequency for *I. floridanum*. This experiment indicates that

roots of both species can persist under a range of irrigation frequencies as long as some portion of the root ball receives some water.

Additional studies were conducted to evaluate the effect of irrigation frequency on horizontal rowth and physiology of *K. latifolia*. In the first experiment, one of three different irrigation frequencies was assigned to all four quadrants of a Horhizotron and consisted of watered-daily or rewatered once substrate percent moisture reached 15% or 10% by volume. Stem water potential and net photosynthesis were measured for the control or once each substrate reached the desired minimum percent moisture. In a second experiment, four different irrigation frequencies randomly assigned among the four quadrants in each Horhizotron and consisted of: watered-daily, unwatered, and rewatered once substrate percent moisture reached 15% or 10% moisture by volume. During the first experiment, HRL was highest in the watered-daily substrate, however, HRL was highest in the unwatered substrate during the second experiment. There were no differences in RDW in either experiment. SDW and GI were highest for plants in the watered-daily substrate in the first experiment. Stem water potential increased with decreasing soil moisture, and net photosynthesis decreased with decreasing soil moisture. These experiments indicate that soil water deficit affects root growth of *K. latifolia* differently depending on if (A) the entire root ball is experiencing soil water deficit, or (B) if only part of the root ball is experiencing soil water deficit. Additionally, stem water potential and net photosynthesis provided good information about the response of *K. latifolia* to different irrigation frequencies.

ACKNOWLEDGEMENTS

The author would like to thank many people who have helped this project to be a success. First and foremost, he would like to thank his family, who have supported him one hundred percent his entire life, in more ways than one. As for leadership, patience, guidance, and most importantly friendship, the author would like to express extreme gratitude and appreciation to Dr. Amy N. Wright. Without her support and encouragement, none of the following would have been possible. For long lasting debates on every subject under the sun, in addition to friendship and insight into this project, the author wishes the thank Dr. Robert C. Ebel. The author would also like to thank Dr. D. Joseph Eakes for insight and willingness to help throughout this project. The author has also met many unique and interesting individuals throughout his two years at Auburn, many of whom will remain close to the author in the future. The author would like to thank his support team: Diana, Julie ('Lil Cleidus), Travis, Kevin, Daniel, and Jeremy for their continued support and close friendship throughout this journey. Without the five o'clock meetings, cook-outs in the trailer park, or Ryan St., this project would not have been a success. The author also wishes to thank anyone in the horticulture department who has had any part in his success.

Style manual or journal used:
HortScience

Computer software used:
Microsoft Word 2003, Microsoft Excel 2003, and SAS v. 9.1

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CHAPTER I

THE EFFECT OF IRRIGATION FREQUENCY ON GROWTH AND PHYSIOLOGY OF NATIVE LANDSCAPE SHRUB SPECIES

Introduction

Research in plant response to water stress is becoming increasingly important, as most climate-change scenarios suggest an increase in aridity in many areas of the globe (Chaves et al., 2003). On a global basis, drought (assumed to be soil and/or atmospheric water deficit) poses an important environmental constraint to plant survival and to crop productivity (Boyer et al., 1982). A better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in horticulture and for forecasting the fate of plants under climate change (Chaves et al., 2003). Interactions between stem water potential, transpiration, photosynthesis, and growth make a study of the effects of water deficits on plant water relations essential and of substantial interest to plant breeders, landscapers, and growers (Ceulemans et al., 1979).

Plant Water

Most woody plants are composed of at least 50% water (fresh weight). Water is important to plants because it serves many different purposes such as the solvent, reactant for specific reactions, and for maintaining turgor (Kramer and Boyer, 1995). As a solvent, water allows gases, minerals, and other solutes to enter and move around a plant in which biochemical reactions occur. When thought of as a reactant, water is catabolyzed during photosynthesis (Kramer and Boyer, 1995). Another essential role of water is the maintenance of turgor, which is important for canopy cell elongation, architecture, opening of stomata, and the movements of leaves, petals, and other plant structures (Kramer and Boyer, 1995).

Water potential is a derivation of chemical potential used to express the free energy status of water in plants and is considered the best measure of plant water status (Boyer, 1969). Water potential is a measure of the amount of work or energy needed to move water between the observed and reference (pure water at ambient pressure and temperature) systems (Kramer, 1966). Absolute chemical potentials are extremely difficult to measure and are not always important to the soil-plant-atmosphere continuum (Spomer, 1985). Instead, the more easily measured difference between chemical potential of water in the system and that of a standard is used when describing a plant's water relations. Units of plant water potential units are a measure of pressure and therefore can be expressed in pascals, bars, or atmospheres. The pascal is the SI unit, however, others have been used in older literature.

Plant water potential has several components, and can be represented by the equation:

$$\Psi_w = \Psi_\pi + \Psi_p + \Psi_g + \Psi_m$$

In this equation, Ψ_w represents total plant or cell water potential, while Ψ_π represents osmotic or solute potential, Ψ_p represents turgor or pressure potential, Ψ_g represent gravitational potential, and Ψ_m represent matric potential. All components of water potential are negative with the exception of pressure potential which can be zero or greater when cells are fully turgid.

Gravitational potential decreases with tree height at a rate of 0.1 MPa per 10 m vertical distance. Matric potential arises from the ability of cell walls or soil particles to hold water to their surfaces (Kramer and Boyer, 1995). It is generally thought that matric potential and gravitational potential do not contribute significantly to plant water potential except in extremely dry tissues and in tall trees, respectively (Abrams, 1988), and thus the equation may be simplified to:

$\Psi_w = \Psi_\pi + \Psi_p$

Osmotic potential arises from dissolved solutes (carbohydrates, alcohols, and mineral nutrients) within plant cells (Kozlowski and Pal lardy, 1997). As total plant water potential decreases, osmotic potential becomes more negative due to an increase in concentration of solutes in plant cells.

Turgor potential comes from xylem tension and positive pressures that come from plant cells as water pushes against cell walls. Stomatal opening and closing and cell elongation are driven by pressure potential (Kozlowski and Pal lardy, 1997). Turgor potential is the only component of total plant water potential that may be positive.

The Cohesion-Tension Theory (C-T theory) is the accepted explanation for the process by which water, or sap, moves from roots to the atmosphere (Canny, 1995). This theory is supported by the fact that water has high cohesive and adhesive forces and forms a continuous column of water within the plant. It can withstand high tensions (low water potentials) of ten to several hundred MPa before the water column breaks (Strudel, 2001). During transpiration, water evaporates from the cell wall surfaces neighboring the intercellular spaces inside a leaf which is replaced by water from inside the cell. As the water moves across the plasma membrane, solutes are left behind. The exiting of water from inside the cell causes the concentration of solutes to increase thereby resulting in a drop in water potential of the cell. A water potential gradient is then formed between all neighboring cells and causes tension within the water bound in the xylem. This tension or pull extends through the continuous column reaching down to the plant roots, causing water to be replaced in the cells that are losing water to the atmosphere. The tension being placed on the roots also lowers their water potential, and water then is drawn from the surrounding soil into the root.

Methods Measuring Plant Water Status

One of the most common instruments used to measure plant water potential is the pressure bomb (Spomer, 1985). An excised leaf or stem and leaf is placed inside an airtight chamber with the cut surface protruding out of the chamber. The pressure bomb applies positive pressure (supplied by compressed gas) to a plant sample that causes the water within the sample to be become visible along the cut surface outside of the pressure bomb (Scholander et al., 1965). When a plant sample is excised, xylem sap withdraws from the cut surface to surrounding cells. When pressure is imposed upon the sample the water moves back into the xylem. When the sap is just visible on the cut surface, the pressure applied equals that of the xylem vessels. After the sap is visible on the cut surface, the reading can be made (Spomer, 1985).

When using the pressure bomb to determine plant water, there are generally three different techniques to use (Chone, 2001). One method is to place a single leaf into the pressure chamber before dawn. When using this technique, it is assumed that plant and soil moisture are equilibrated just before dawn (Sellin, 1998), therefore pre-dawn water potential measurements are used in ecophysiological studies as a measure of moisture at the root surface. This method is used much, but there is debate as to its validity because some species need to transpire during the night not allowing the equilibrium to be reached (Ceulemans et al., 1988). Daily leaf water potential (taken after sunrise) measures local leaf water demand, and if several samples are taken on a plant, can reflect plant water potential. Several samples must be taken because leaf water potential (after sunrise) is extremely variable and factors such as leaf age, angle to the sun, position in the canopy, growth stage, and environment (field/controlled) must be accounted by the researcher (O’Toole and Cruz, 1980; Sionit and Kramer, 1976; Turner and Begg, 1973). The third method is to measure water potential of a non-transpiring leaf as an estimate of water potential (Begg and Turner, 1970). This is able to be achieved by wrapping a leaf with plastic and aluminum foil and allowing at least one hour to pass before taking the reading (Begg and Turner, 1970). This allows leaf water potential to equal stem water potential. Stem water potential is the result of whole plant transpiration and indicates the plant’s ability to move water from the roots to the atmosphere (Chone, 2001). Past studies have shown stem water potential to be a good indicator of water deficit since readings are independent of factors such as leaf age, angle to the sun, position in the canopy etc. (Garnier and Berger, 1985).

Photosynthesis

Stomata are key components of plant anatomy because stomata facilitate carbon dioxide intake and water loss in the form of water vapor by leaves. Stomatal closure is one of the first lines of defense against desiccation (Yordanov et al., 2000, Chaves et al., 2003). Plants close stomata in order to equilibrate transpirational water loss through the leaf surfaces with the absorption rate of roots (Hopkins, 1995). The closing of stomata results in a reduced ability in the efficiency of leaves to fix carbon, lower leaf formation/expansion, and the shedding of leaves (Kozlowski and Pallardy, 1997).

Stomata opening/closing are controlled by turgor pressure. An increase in turgor pressure in guard cells causes stomata to open, while a decrease in turgor pressure in guard cells results in closed stomata (Chaves et al., 2003). Once stomata close in response low soil and plant water potential, carbon dioxide may not be available in sufficient amounts to satisfy the photosynthetic machinery of a plant, resulting in a decrease in carbon assimilation (Yordanov et al. 2000). This phenomenon may result from shoot dehydration (Chaves, 1991), while other studies suggest root dehydration is responsible and is explained by root signaling (Davies, 2003).

Restricted stomatal aperture and subsequent leaf growth may decrease water loss as a result of chemical signals generated by roots in relation to drying soil (Zhang and Davies, 1989). These chemical signals are produced in roots and are thought to move through the xylem stream to communicate to the shoots the water status near the roots (Trejo et al., 1993). Many researchers believe that this phenomenon is attributed to the plant hormone abscisic acid (ABA) (Gowing et al., 1990; Hopkins, 1995; Davies et al., 2003). ABA's specific role has yet to be proven conclusively (Chaves, 2003).

In order to demonstrate this phenomenon, one study utilized a split root technique (half of the root system was well watered, and half of the root system was exposed to drying soil) and observed a decline in leaf expansion, leaf initiation, and stomatal aperture, while there was no effect on shoot water relations (Gowing et al., 1990).

Photosynthesis can be measured with the use of LiCor 6400 Photosynthesis machine. This type of photosynthesis machine is an open steady state system, and is frequently used to measure a single leaf's gas exchange (Bugbee, 1989). Measurements of this type are usually measured in the unit micromoles CO_2/ meter^2/second. This machine works by measuring the carbon dioxide in the air inside the cuvette and then compares it to a reference. An Infrared Gas Analyzer, or IRGA, facilitates the measurement of the carbon dioxide concentration. A cuvette is clamped onto a leaf and then carbon dioxide is passed over the leaf. Next, the machine compares the amount of carbon dioxide of the sample inside the cuvette to that of the reference that has not been exposed to the leaf. The difference in the two carbon dioxide concentrations represents the net photosynthesis of the leaf at that time (Mitchell, 1992). This machine also utilizes a light to set specific amounts of photosynthetically active radiation (PAR). Using this feature allows a researcher to measure net photosynthesis for any amount of sunlight throughout the day.

Methods of Inducing Water Deficit

There are several ways to simulate drought conditions in a controlled environment. The most simple and natural method for inducing plant moisture stress is by withholding irrigation (Krizek, 1985) or reducing the frequency of irrigation (Whalley et al., 2000). When employing either of these methods, it is important to account for container size and soil type (Whalley et al., 2000). For a gradual increase in drought, a larger container containing a substrate that dries slowly would be better than a small container containing a substrate that dries rapidly. The length of time the water is withheld and/or the amount of water withheld is up to the researcher. With either method, once a desired minimum is achieved, the substrate can be rewatered and another drying cycle can be induced (Zwack et al., 1999). Controlling irrigation frequency is very popular, but this process often allows water status to decline at such a fast rate that plants may wilt before any symptoms are evident (Fan, 1997), but if proper container size and substrate are chosen, this will not be an issue (Griffin et al., 2004). Another technique for conducting drought experiments is to try to maintain a constant level of drought by replacing the amount of water lost due to evapotranspiration. This method is achieved by determining container mass while at field capacity then weighing the container later to determine the amount of water lost and applying the amount lost to the soil or substrate (Zwack et al., 1998). Disadvantages of this method are that the amount of water added is usually small and will not penetrate the entire rhizosphere, resulting in an uneven distribution of root growth. Additionally the mass of the growing plant is often not accounted for leading many to misinterpret the weight of the pot.

When conducting an experiment involving the control of irrigation frequency, several different instruments can be used to estimate soil water content. Researchers can choose from soil tensiometers, psychrometers, resistance blocks, neutron probes, soil xylem water potential (pre-dawn), or gravimetric water content. Volumetric soil moisture content based on apparent dielectric constant can be measured with TDR (time domain reflectometry) devices. TDR works by releasing an electric pulse into the substrate from one electrode and measuring the current by another electrode. Since water conducts electricity, the time to retrieval of the electric pulse will be quicker the more moisture there is in the substrate or soil.

Application of polyethylene glycol (PEG) to soil, substrate, or hydroponically is another means to simulate drought conditions. PEG causes water stress by reducing the matric potential of substrates by forming hydrogen bonds with water resulting in reduced amounts of plant available water (Kjellander and Florin, 1981; Steuter, 1981). Researchers use PEG because it is a more precise way, than the above mentioned, to control soil water potential (Fan, 1997). PEG is available in different molecular weights (600-20,000 g/mole), and it has been noted that the heavier versions (8000 and higher) are less toxic to plants since they are not as readily absorbed by roots (Burnett et al., 2006). The use of PEG must be used with caution because plant injuries caused by absorption of PEG can be easily confused with injuries suffered through drought (Whalley et al., 2000).

Importance of Root Growth in Landscape Establishment

Root growth following transplanting is one of the most vital factors affecting post-transplant establishment of container-grown plants (Watson and Himelick, 1997), since having a healthy, functioning root system is imperative for plant productivity and survival of land plants (Wraith and Wright, 1998). Woods (1959) found that the time to initiation of new root growth is important for transplant success, since initiation of new root growth after transplanting has been associated with successful transplant establishment into the landscape. This root growth following transplanting allows plants to obtain water and nutrients needed for survival. Roots are not only important for the uptake of water and nutrients, but are also necessary for anchorage, storage of carbohydrates, and synthesis of certain hormones (Kozlowski and Pallardy, 1997).

Root growth frequently decreases during soil drying (Davies and Bacon, 2003; Keys and Grier, 1980),which can be attributed to a decrease in root cell volume and cell turgor (Nepomuceno et al., 1998). Without turgor, cell elongation is halted and growth stops (Davies, 2003). Geotropism is another common response to soil drying (Sharp et al., 1985). In an experiment evaluating rooting depth, researchers have found that the longest vertical root lengths occurred in unwatered treatments (Reader et al., 1993; Klepper, 1973). Drought can also affect the diameter of roots. Roots in soil with low water potential and low mechanical impedance are thinner than roots in a well-watered soil (Davies and Bacon, 2003). Sharp et al. (1988) suggests plants with this root pattern are able to allocate limited carbohydrate supply to root length rather than width in order to explore deeper territory. On the other hand, roots may also be thicker when moisture is limiting (Spollen et al., 2000). This occurrence is thought to aide roots in penetrating soil with high mechanical impedance more easily (Davies and Bacon, 2003). The shape, size, and pattern of roots are also species dependent.

Root Observation

There are many methods for studying root systems and measuring root growth, however most are labor intensive and/or expensive (Wright and Wright, 2004). *Rhizotron* originates from the Greek words *rhizos* meaning root and *tron* meaning instrument, and may be described as a fixed building or structure used to study or view underground plant parts through clear surfaces (Klepper and Kaspar, 1994). These structures allow researchers the opportunity to view root and shoot growth simultaneously. Rhizotrons are permanent structures that consist of transparent walls positioned against a native soil type or other soil types from other regions (Klepper and Kaspar, 1994). There are not many rhizotrons throughout the world because of the large capital investment, difficulty of construction, and the diverse research teams needed to properly utilize the structure (Huck and Taylor, 1982; Klepper and Kaspar, 1994). A more convenient way to measure root growth than the traditional rhizotron is the minirhizotron which consists of tubes inserted into the ground into which cameras are inserted to view roots. Minirhizotrons also negatively affect the pattern of natural root growth as roots frequently follow the camera tubes installed. Installation of camera tubes also can drastically affect the physical properties of the soil (Taylor et al., 1990). Root dyes have been used to quantify root growth over time. With this method a transplant is drenched with a colored dye, typically red, blue, or yellow. The roots present at the drenching will then stay the color of the dye, while new roots will either be white or brown (due to suberization) (Arnold and Young, 1990; Wright and Wright, 2004). This technique is generally used for short-term studies since many woody species roots suberize, making the dye useless).

The Horhizotron™ (horizontal root growth measurement instrument), a type of spilt root technique and a container-type rhizotron, is a fairly new research tool being used to studying root growth. It utilizes eight panes of glass that extend away from the root ball of a contain-grown plant, to form four wedge-shaped quadrants (Wright and Wright, 2004). The quadrants are 10.5 inches deep and are filled with substrate, and different substrates or treatments may be applied to each quadrant. The quadrants are secured upon a square 2' x 2' piece of aluminum. To exclude light from the root zone, the root ball and quadrants are enclosed in a box constructed of ¾ inch foam insulation board. Root length data can be recorded periodically (frequency depends on rate of root growth) until the root apices meet the end of the quadrant (25 cm). It is unique in that it helps researchers to measure horizontal root growth (representing post-transplant root exploration into the surrounding substrate) continuously over time and not just vertical root growth. Researchers are also able to the see the time from transplant to new root growth. It also allows for changes in chemical and physical composition among the four quadrants or plants can be subjected to the same treatment in each quadrant. The Horhizotron is especially beneficial because it can be used indoors or outdoors, and it is lightweight, portable, and inexpensive to construct. The Horhizotron has not yet been used for a drought study, but other split root techniques have been used successfully for drought studies (Coutts, 1982).

<u>Native Plants in the Landscape</u>

The concept of using native plants in the landscape stems from a movement in landscape design called the naturalized landscape. The thought behind a naturalized landscape is that plants native to a certain area will be specifically adapted to the conditions of that area and will require less additional inputs, such as water, fertilizer, herbicides, etc (Alder and Ostler, 1989). The increase in the use of native plants in the last 10-20 years has shown increased cost savings in maintenance, pruning, and materials, as well as a great transition to the surrounding landscape (Alder and Ostler, 1989). The increased interest in native landscape species has encouraged large wholesale nurseries to increase production of native plants and has led to landscape architects and designers including native plants in their plans. Interest in native plant use will encourage interest in expanded plant availability.

When designing a landscape for native plant species, it is important to consider many aspects of the site, including soil type, rainfall, slope, exposure, elevation, competition, and microclimates (Alder and Ostler, 1989). Correct selection of plant material must also be taken into consideration. When selecting plants species such factors as tolerance to site stress (wind, temperature, drought, and sun exposure), soil conditions (salinity, water holding capacity, texture, and depth), growth form, and life expectancy should also be considered. If all of these aspects are considered, along with appropriate cultural practices, it can be possible to successfully implement a naturalized landscape and costs associated with the landscape will decline. With the use of native plants water requirements will also decline. With increases in aridity and water restrictions imposed by municipalities, it is of considerable interest to implement the use of more drought tolerant shrubs to reduce water usage for the homeowner (Alder and Ostler, 1989).

Morella cerifera (L.) Small (syn. *Myrica cerifera*) (wax myrtle) is a broad leaved evergreen shrub that can grow to a height of 9 m (Dirr, 1998). *M. cerifera* usage in the landscape consists of pruned screens and as a small tree. *M. cerifera* has the ability to grow in full sun or part shade and is tolerant of salt spray. It is also known to grow in a variety of harsh environments, such as growing in slightly brackish waters, bogs, upland woods, and old fields. The westward portion of its geographic distribution extends from eastern Texas to South America, and the eastern part from southern New Jersey to the Florida Keys. USDA hardiness zones range from 8-11.

Illicium floridanum Ellis (Florida anise tree) is a broad leaved evergreen shrub that can grow up to 3 m (Dirr, 1998). Landscape attributes include a pleasing odor and dark green foliage that blends nicely into naturalized plantings. Also of interest is the plant’s disease resistance and that it produces certain compounds in the leaves that deter many insects. *I. floridanum* typically prefers moist well-drained soils and shade, but has been observed thriving in inundated areas. The westward portion of its geographic distribution extends to Louisiana, and the eastern part extends to Florida. USDA hardiness zones range from 6-9.

Kalmia latifolia L. (Mountain laurel) is a native broad leaved evergreen shrub capable of growing up to 15 m. *K. latifolia* is considered once of the most prized native shrubs for landscaping (Dirr, 1998). *K. latifolia* can be planted in mass near shady borders, full sun, or in rocky soils. Most notable about this species is the floral display. The flowers range from white to almost purple. Even though *K. latifolia* is prized by many plant enthusiasts, it frequently does not survive transplant into the landscape. The westward portion of its geographic distribution extends to Ohio, and the east part extends from Quebec to Florida. Wright et al. (2003) conducted work to compare the species drought tolerance with that of Japanese holly, and that *K. latifolia*'s poor performance in the landscape may be due to its relatively low drought tolerance and its slow rate of root growth (Wright et al, 2004).

Summary

Population growth and increases in long-term drought have made a conscious public more aware of the benefits, such as drought tolerance, less maintenance, and none are invasive, of native plant species (Meyer, 2005). Characterizing root growth responses to drought for native plant species could produce recommendations for drought tolerant plants. Research into the three species described might also suggest irrigation recommendations for successful landscape establishment. The objectives of this study were (1) to determine the effect of drought on root growth of wax myrtle and Florida anise tree, and (2) determine the effect of drought on root growth, photosynthesis, and stem water potential of mountain laurel.

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CHAPTER 2

THE EFFECT OF IRRIGATION FREQUENCY ON POST-TRANSPLANT ROOT GROWTH OF *MORELLA CERIFERA* AND *ILLICIUM FLORIDANUM*

Abstract.

Knowledge of plant responses to drought can provide information pertaining to relative drought tolerance and irrigation requirements of native landscape shrub species. This study utilized the Horhizotron™ (modified split-root, container-type rhizotron) to simulate post-transplant root growth into four surrounding substrates with different irrigation frequencies. The Horhizotron contained of eight glass panes arranged to form four quadrants extending away from the original container root ball. Plants of *Morella cerifera* (L.) Small (syn. *Myrica cerifera*) (wax myrtle) and *Illicium floridanum* Ellis were planted into Horhizotrons on 9 Feb. and 15 June 2006, respectively, in a greenhouse in Auburn, AL. Each quadrants was filled with Greens Grade™ Profile™, a fine textured calcined clay material amended with 9.5 kg·yd^{-3} 17N-5P$_2$O$_5$-11K$_2$0, 1.2 kg·m^{-3} dolomitic limestone, and 0.9 kg·m^{-3} Micromax™. Four different irrigation frequencies were randomly assigned among the four quadrants in each Horhizotron and consisted of: watered daily, unwatered, and watered once 10% or 15% moisture by volume was reached. Root balls of all plants received 400 mL of water daily and when watered, quadrants received 400 mL water. Weekly horizontal root length (HRL) was recorded by measuring the five longest roots (measured parallel to the ground) visible on each glass pane of a quadrant. The experiment was repeated for both species beginning 15 Mar. 2007. Root dry weight for the first experiment were determined for *M. cerifera* and *I. floridanum* 79 and 107 days after planting (DAP), respectively, and for on 51 and 111 DAP, respectively in the second experiment. In the first experiment, *M. cerifera* and *I. floridanum* HRL decreased with decreasing irrigation frequency as did root dry weights. In the second experiment no difference in HRL or root dry weights was seen among treatments for *M. cerifera* however, HRL and root dry weight decreased with decreasing irrigation frequency for *I. floridanum*. Although root lengths and dry weights were lower in the quadrants receiving less frequent irrigation, root growth still occurred in these quadrants. This experiment indicates that roots of both species can persist under a range of irrigation frequencies as long as some portion of the root ball receives some water.

Introduction

Poor appearance, dieback, or even death of container grown woody landscape plants transplanted into the landscape can be frequently attributed to water stress (Beeson, 1994; Costello and Paul, 1975). Newly transplanted landscape species are removed from an environment of daily irrigation and planted into the landscape where irrigation is often far less frequent. This reduction in irrigation frequency in addition to the pull of the surrounding landscape soil on water in the root ball can cause severe water deficits within the root ball. Therefore, it is suggested that irrigation should be higher than what the container received in production, until roots grow out from the original container root ball into the surrounding soil (Costello and Paul, 1975).

Research into plant response to water stress is becoming increasingly important, as most climate-change scenarios suggest an increase in aridity in many areas of the globe (Chaves et al., 2003). On a global basis, drought (assumed to be soil and/or atmospheric water deficit) poses important environmental constraints to plant survival and to crop productivity (Boyer et al., 1982). A better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in horticulture and for forecasting the fate of plants under climate change (Chaves et al., 2003).

One possible strategy to combat increasing drought is to implement the use of native plant species. In recent years, the popularity of these plants has increased dramatically. Many believe that plants (of a certain location) are more adapted to live in that environment and should be used to replace landscape plants with high water requirements (Meyer, 2005). With increases in aridity and water restrictions imposed by municipalities, it is of considerable interest to implement the use of more drought tolerant shrubs to reduce water usage for the homeowner (Alder and Ostler, 1989).

A healthy, functioning root system is imperative for plant productivity and survival of land plants (Wraith and Wright, 1998). Root growth following transplanting is one of the most crucial factors affecting post-transplant establishment of container-grown plants (Watson and Himelick, 1982). Growth of roots into the surrounding soil is crucial for a plant to attain the required elements needed for survival, most notably water and mineral nutrients.

Quantifying lateral root growth from the original container root ball into the surrounding soil is important since time to new root growth is correlated with plant establishment (Woods, 1959). Observation of post transplant root growth can be very indicative of root growth preference among rhizosphere conditions. Several techniques have been used in the past to study post transplant root growth including rhizotrons, minirhizotrons, and root dyes. Rhizotrons are permanently fixed structures that involve a large capital investment and only allow for vertical root growth observation (Huck and Taylor, 1982). Minirhizotrons consist of small cameras that are inserted into tubes placed in the ground and require a costly initial investment. Plants may also be dipped into dyes, causing original roots to remain the color of the dye, in order to evaluate root growth. A new technique, the Horhizotron™, provides an inexpensive, lightweight, and simple method of measuring root growth over time (Wright and Wright, 2004). This technique is non-destructive and provides an easy method for quantifying root growth in various rhizosphere conditions. The Horhizotron allows up to four different rhizosphere conditions to be imposed upon a single plant and allows researchers to quantify post-transplant root growth from the original root ball into the surrounding substrate.

Morella cerifera (L.) Small (syn. *Myrica cerifera*) (wax myrtle) is a broad-leaved evergreen shrub that can grow to a height of 9 m (Dirr, 1998). *M. cerifera* usage in the landscape consists of pruned screens and as a small tree. *M. cerifera* has the ability to grow in full sun or part shade and is tolerant of salt spray. It is also known to grow in a variety of harsh environments, such as growing in slightly brackish waters, bogs, upland woods, and old fields. The westward portion of its geographic distribution extends from eastern Texas to South America, and the eastern part from southern New Jersey to the Florida Keys. USDA hardiness zones range from 8-11.

Illicium floridanum Ellis (Florida anise tree) is a broad-leaved evergreen shrub that can grow up to 3 m (Dirr, 1998). Landscape attributes include a pleasing odor and dark green foliage that blends nicely into naturalized plantings. Also of interest is the plant's disease resistance and that it produces certain compounds in the leaves that deter many insects. *I. floridanum* typically prefers moist well-drained soils and shade, but has been observed thriving in inundated areas. The westward portion of its geographic distribution extends from northern Mississippi to southern Louisiana, and the eastern part extends from northern Georgia to southern Florida. USDA hardiness zones range from 6-9.

Both *M. cerifera* and *I. floridanum* are native plants to the southeast, where this research was conducted. *M. cerifera* and *I. floridanum* are plants of differing habitats, thus drought may affect root physiology differently for these two species. The objective of this study was to compare and contrast the effect of irrigation frequency on post-transplant horizontal root growth of these two species of differing habitats.

Materials and Methods

Experiment 1. On 9 Feb. 2006 and 15 June 2006, 5 plants each of both *M. cerifera* and *I. floridanum*, respectively, were removed from 11.3 L containers, and, each plant was placed in the center of a single Horhizotron (Wright and Wright, 2004). Each Horhizotron was placed on a greenhouse bench at the Patterson Horticulture Greenhouse Complex, Auburn University, Auburn, Ala. (average day/night temperatures set at 26/21°C). Each Horhizotron contained four wedge-shaped quadrants, each constructed from 2 glass panes measuring 26 cm in length and 20 cm in height, extending away from the root ball of each plant. Quadrants were filled with Greens Grade™ Profile™ (Profile Products, Buffalo Grove, Ill.), a calcined clay material, that had been amended with 9.5 $kg{\cdot}yd^{-3}$ 17N-5P_2O_5-11K_2O) (Pursell Industries, Sylacauga, Ala.), 1.2 $kg{\cdot}m^{-3}$ dolomitic limestone, and 0.9 $kg{\cdot}m^{-3}$ Micromax™ (The Scotts Co., Marysville, Ohio). Greens Grade Profile was chosen because its chemical and physical properties are similar to field soil and upon completion of the study, is easily removed from roots for determining root dry weight. Substrate in all quadrants in each Horhizotron and original plant root balls received daily irrigation until roots had grown about five centimeters into each quadrant at which time treatments were initiated. Treatments were four different irrigation frequencies randomly assigned to the four quadrants within each Horhizotron. Treatments included: substrate watered daily (~20% moisture by volume), substrate rewatered once dried to 15% moisture, substrate rewatered once dried to 10% moisture, and substrate that remained unwatered throughout the study. Treatments for *M.*

cerifera and *I. floridanum* were initiated on 8 Mar. 2006 (28 days after planting, DAP) and 28 June 2006 (14 DAP), respectively. Substrate percent moisture (by volume) was measured daily using a Theta probe (Delta-T Devices Ltd., Cambridge, England). When watered, quadrants received 400 mL water. Daily, 400 mL water was also applied directly to the original container root ball.

Weekly root growth measurements were taken by measuring the horizontal root length (measured parallel to ground, HRL) of the five longest roots visible along the glass panes that formed each side of a quadrant (two glass panes per quadrant). At the end of the study, roots that had grown into each quadrant were cut from the original root ball, and the substrate was gently rinsed from those roots. These roots were then dried for 48 hours at 66 °C, and root dry weights (RDW) were recorded. Initial and final plant growth indices were also measured.

Experiment 2. The above experiment was repeated with both plant species being planted on 15 Mar. 2007. The experimental design and all treatments were identical to the first experiment with the exception of the amount irrigation applied. When the desired substrate percent moisture was achieved during the second experiment, quadrants received 600 mL of water. The substrate watered daily and the original container root ball received 600 mL water at each watering. The amount of irrigation was increased to keep plants from wilting because the plant material was larger than the first experiment.

The experimental design, for both experiments, was a randomized complete block design, with five blocks per species and one Horhizotron equaling one block. Data were analyzed using General Linear Model procedures and regression analysis, and means were separated using Least Significant Difference (*P*<0.05) (SAS, 2004). Species were analyzed separately in the first experiment, however, in the second experiment; species was included in the model.

Results

Morella cerifera. Horizontal root length (HRL) during the first and second experiments increased linearly over time for all treatments throughout the experiment (Table 2; Figure 1A & 1B). Differences in HRL among treatments during the first experiment were first noticed 64 DAP. On this day HRL in the watered daily control, the rewatered at 15% moisture, and the rewatered at 10% moisture were higher than in the substrate that remained unwatered. On 70 DAP, HRL was highest in the watered daily control and the substrate rewatered at 15% moisture, followed by HRL in the substrate rewatered at 10% moisture with HRL being lowest in the substrate that remain unwatered (Fig. 2A). Root dry weights (RDW) for all treatments followed the same trend as HRL (Fig. 3A).

During the second experiment, 43 DAP was the first date that differences in HRL were noticed. On this day the substrate that remained unwatered, the well-watered control, and the substrate rewatered at 10% moisture had higher HRL than in the substrate rewatered at 15% moisture. By the end of the experiment (48 DAP) HRL in the substrate rewatered at 10% moisture and the unwatered substrate were higher than the substrate rewatered at 15% moisture and the watered daily control. Even though HRL in the substrate rewatered at

10% moisture and HRL in the unwatered substrate were higher than the other two substrates, the means were so close of the throughout the experiment, it is doubtful there is actually any biological significance to this result, especially when *I. floridanum* HRL results are considered. However, RDW in the watered daily control and the substrate that remained unwatered were similar and higher than that in the substrate rewatered at 15% moisture and the substrate rewatered at 10% moisture were (Fig. 3B).

During both experiments with *M. cerifera,* the length of time between irrigation applications for the substrate rewatered at 15% moisture and the substrate rewatered at 10% moisture decreased with time. At the beginning of both experiments the substrate rewatered at 15% moisture usually took about two to three days to reach the desired minimum, while the substrate rewatered at 10% moisture usually took about five to six days. Towards the end of each experiment, the substrate rewatered at 15% moisture reached the desired minimum almost daily, while the substrate rewatered at 10% moisture reached the desired minimum usually within two to three days of the previous irrigation event.

Illicium floridanum. HRL in both experiments increased linearly for the watered daily substrate and the substrate that was rewatered at 15% moisture, while HRL in the substrate rewatered at 10% moisture and in the substrate that remained unwatered increased quadratically (Table 3; Fig. 2A & 2B). During the first experiment at 44 DAP HRL in the watered daily control and in the substrate rewatered at 15% moisture was similar and higher than the HRL in the substrate rewatered at 10% moisture and in the substrate that remained unwatered. By the end of the experiment (107 DAP) HRL for the watered daily control and the substrate rewatered at 15% moisture was similar and higher than in the substrate rewatered at 10% moisture, which was higher than in the substrate that remained unwatered (Fig.2A). Root dry weight data also followed a similar trend, being higher in the watered daily control and the substrate rewatered at 15% moisture in the substrate rewatered at 10% moisture and in the substrate that remained unwatered (Fig. 5A).

During the second experiment HRL at 56 DAP in the watered daily control was higher than HRL in the other three substrates. At 79 DAP a similar trend to the first experiment was recognized with HRL in the watered daily control being highest followed by HRL in the substrate rewatered at 15% moisture, the substrate rewatered at 10% moisture, and the substrate that remained unwatered. By 110 DAP HRL in the water daily control was highest, followed by HRL in the substrate rewatered at 15% moisture, with HRL being lowest in the substrate rewatered at 10% moisture and in the substrate that remained unwatered (Fig. 2B). RDW followed a similar trend to HRL with RDW in the watered daily control and in the substrate rewatered at 15% moisture being similar and higher than the values for the substrate rewatered at 10% moisture and the substrate that remain unwatered (Fig. 5B).

During both experiments, the length of time between irrigation applications decreased with time for the substrate rewatered at 15% moisture. Toward the beginning of both experiments irrigation events happened usually within two or three days from the previous irrigation event, while toward the end of the experiment irrigation events happened almost daily, length of time between irrigation events in the substrate rewatered at 10% remained consistent throughout the experiment.

Discussion

Morella cerifera. More root growth in substrates that contained higher levels of soil moisture is a common response, for some species, in spilt root technique studies (Coutts, 1982). Even though HRL of *M. cerifera* in the substrate rewatered at 10% moisture and in the substrate that remained unwatered was less than in the other two substrates, root growth still occurred. Root growth was not halted due to drier treatments in both experiments, so it could be assumed that plants in both experiments may have been allocating water resources from areas of higher moisture content within the root to the areas where moisture was limiting, a process known as hydraulic redistribution (Coutts, 1982; Warren et al., 2007). Applying water to part of a root system may equalize root xylem potential, and longitudinal resistance to water is frequently small (Slavikova, 1967), thus allowing relatively unimpeded movement of water across the root system. The transfer of water within the root system is probably due to a water potential gradient which is formed, and is thought to enable plants to continue growth, maintain turgor, and absorb nutrients necessary for development (Breazeale, 1930),. Other studies involving *M. cerifera* have been conducted comparing the hydraulic properties of the plant with that of fast spreading invasive plant species, and results indicated that *M. cerifera* has excellent hydraulic properties (resistance to cavitation) (Pratt and Black, 2006), a feature that probably allows *M. cerifera* to thrive in upland areas of its native habitat.

Illicium floridanum.

I. floridanum in both studies had substantially more root growth in the watered daily control and in the substrate rewatered at 15% moisture than in the other two drier treatments. This would indicate the plant was not as able as *M. cerifera* to hydraulically redistribute water within its root system in order to allow all roots, within its root system, to explore or discover other water resources. This could be due in part to the plant's native habitat which is a moist to almost wet environment (low lying areas and swamps) (Dirr, 1998). This plant may instead be able to display hydrotropic properties, or the ability to grow and proliferate towards areas of higher moisture content (Eapen, et al., 2005).

Both *M. cerifera* and I*. floridanum* exhibited sustained root growth, but *M. cerifera* had higher rates of root growth than *I. floridanum* (Tables 2 and 3). *M. cerifera* took an average 37 DAP for root in the well watered control to reach the end of the quadrant, while HRL in the well watered control for *I. floridanum* took an average of 108 DAP to reach the end of the quadrant. The higher rate of root growth for *M. cerifera,* post-transplant, could facilitate more water absorption from different water resources, thus allowing the plant to establish in the landscape more quickly than *I. floridanum*, especially when water is limiting.

Based on this experiment, *M. cerifera* would be an excellent choice for placement in a landscape situation where water is limiting, as long as some portion of the root system is receiving some water. *M. cerifera's* ability to continue root growth in all directions, even in dry portions of the rhizoshere, and possibly its hydraulic redistribution properties, will most likely improve the plant's chances of discovering other water resources and lead to overall plant establishment. Even though *I. floridanum* did not display as much root growth as *M. cerifera*, some root growth still occurred. When placing *I. floridanum* in the landscape, it may be necessary to pay closer attention to post-transplant irrigation frequency than *M. cerifera*.

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Table 1. Significance of main effects and interactions for treatment[z], block, species[y], and harvest[x] during experiment two.

Significance	P-value
Block	<0.0001
Treatment	<0.0001
Block*Treatment	<0.0001
Species	<0.0001
Species*Treatment	0.6360
Harvest	<0.0001
Treatment*Harvest	0.1964
Species*Harvest	<0.0001
Treatment*Species*Harvest	<0.0001

[z] Irrigation Frequency

[y] *M. cerifera or I. floridanum*

[x] Weekly root growth measurements (measured parallel to the ground)

Table 2. Effect of irrigation frequency (treatment) on final horizontal root length (HRL^{z}) of *M. cerifera* growing in Horhizotrons in Auburn, AL, regression equations for change in HRL over time with corresponding R^{2} term and significance of regression equation (P-value), and significance of treatment main effects and interactions for HRL. Plants were grown in greenhouse in a greenhouse in Auburn, AL.

Experiment 1, 70 DAP, 9 Feb - 28 April, 2006.					Experiment 2, 48 DAP, 15 Mar - 5 May, 2007.				
Treatment	HRL (cm)	Equation^{x}	R^{2}	P-value	Treatment	HRL (cm)	Equation	R^{2}	P-value
Watered Daily	25.82 a^{w}	y = 0.47x - 7.00	0.73	<0.0001	Watered Daily	20.14 b	y = 0.61x - 9.34	0.62	<0.0001
15%^{v}	25.72 a	y = 0.52x - 10.30	0.80	<0.0001	15%	19.88 b	y = 0.58x - 8.01	0.10	<0.0001
10%^{u}	24.26 b	y = 0.71x - 12.76	0.70	<0.0001	10%	22.76 a	y = 0.71x - 12.76	0.68	<0.0001
Unwatered	21.54 c	y = 0.44x - 7.53	0.59	<0.0001	Unwatered	21.32 a	y =0 .62x - 9.53	0.56	<0.0001
Significance	P-value				P-value				
Treatment	0.6505				0.9800				
DAP	<0.0001				<0.0001				
Treatment x DAP	<0.0001				0.6500				

[z] HRL = root length measured parallel to the ground

[y] DAP = days after planting in Horhizotron

[x] y = HRL, x = DAP

[w] Lowercase letters denote mean separation (n = 50) among treatments by LSD at $P < 0.05$ (SAS, 2004)

[v] Quadrants were rewatered at 15% moisture determined with a Theta Probe

[u] Quadrants were rewatered at 10% moisture determined with a Theta Probe

Table 3. Effect of irrigation frequency (treatment) on final horizontal root length (HRLz) of *I. floridanum* growing in Horhizotrons in Auburn, AL, regression equations for change in HRL over time with corresponding R2 term and significance of regression equation (P-value), and significance of treatment main effects and interactions for HRL. Plants were grown in greenhouse in a greenhouse in Auburn, AL.

Experiment 1, 107 DAP, 15 June - 20 Sept, 2006.					Experiment 2, 110 DAP, 15 Mar - 10 June, 2007.				
Treatment	HRL (cm)	Equation[x]	R^2	P-value	Treatment	HRL (cm)	Equation	R^2	P-value
Watered Daily	24.9 a[w]	$y = 0.22x + 2.08$	0.93	<0.0001	Watered Daily	17.64 a	$y = 0.25x - 9.91$	0.85	<0.0001
15%[v]	25.1 a	$y = 0.23x + 1.67$	0.54	<0.0001	15%	16.42 b	$y = 0.24x - 10.05$	0.54	<0.0001
10%[u]	16.6 b	$y = -0.001x^2 + 0.21x + 2.92$	0.64	<0.0001	10%	11.16 c	$y = -0.003x^2 + 0.62x - 22.22$	0.70	<0.0001
Unwatered	15.8 c	$y = -0.001x^2 + 0.28x + 1.18$	0.75	<0.0001	Unwatered	10.76 c	$y = -0.003x^2 + 0.56x -20.57$	0.70	<0.0001
Significance	P-value				P-value				
Treatment	<0.0001				0.0002				
DAP	<0.0001				<0.0001				
Treatment x DAP	<0.0001				<0.0001				

[z] HRL = root length measured parallel to the ground

[y] DAP = days after planting in Horhizotron

[x] y = HRL, x = DAP

[w] Lowercase letters denote mean separation (n=50) among treatments by LSD at $P < 0.05$ (SAS, 2004)

[v] Quadrants were rewatered at 15% moisture determined with a Theta Probe

[u] Quadrants were rewatered at 10% moisture determined with a Theta Probe

Figure 1: (A) Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *M.cerifera* grown from February 8 – April 28 in Horhizotrons in a greenhouse. Treatments began 14 days after planting (DAP). HRL increased linearly for the substrate water daily, substrate rewatered once dried to 15%, substrate rewatered once dried to 10% and the unwatered substrate (*P*<0.0001). (B) Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *M.cerifera* grown from March 15 – May 1 in Horhizotrons in a greenhouse. Treatments began 30 days after planting (DAP). HRL increased linearly for substrate water daily, substrate rewatered once dried to 15%, substrate rewatered at 10% and the unwatered substrate (*P*<0.0001), Points are means of 50 observations.

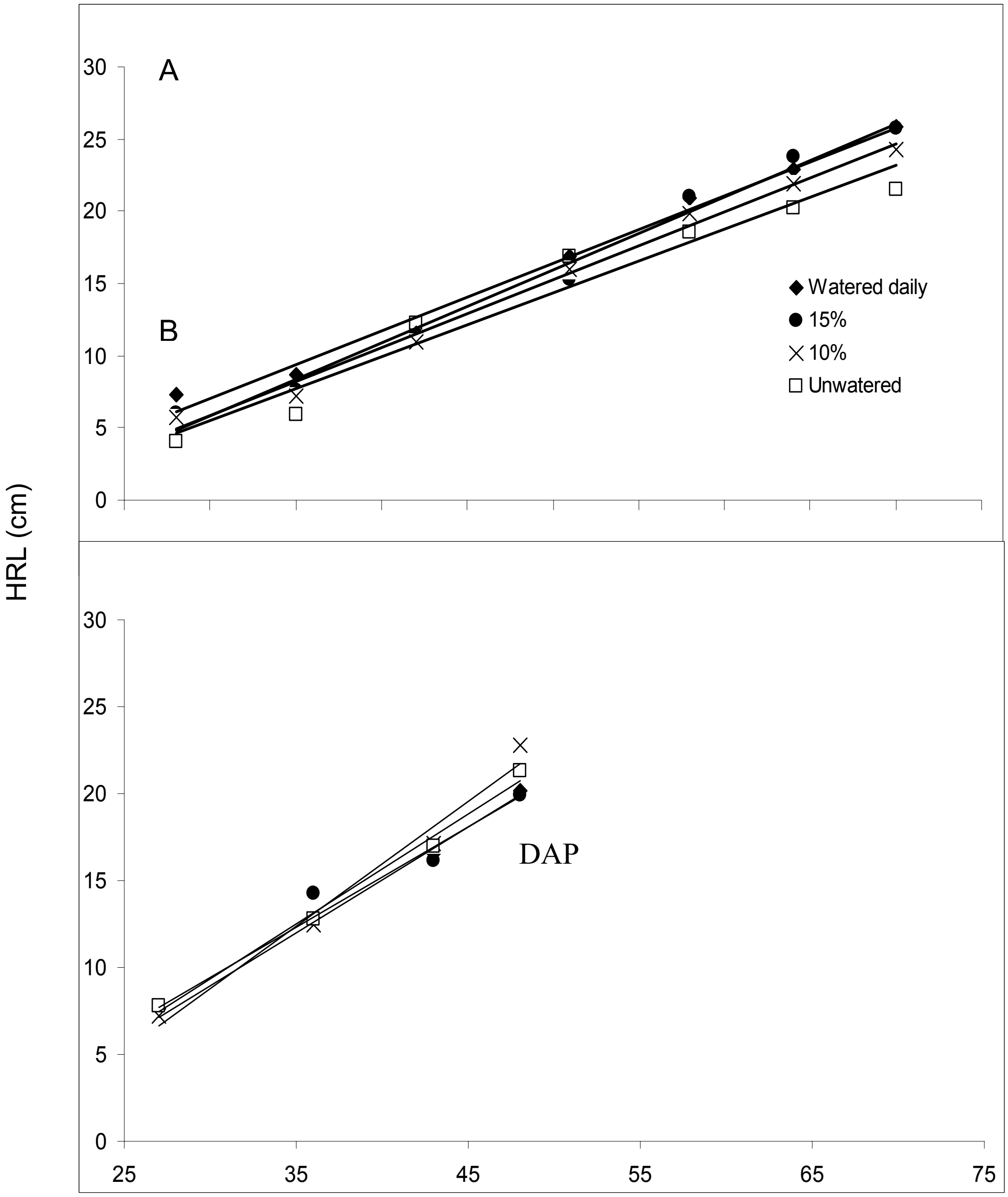


Figure 2: Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *I. floridanum* grown from (A) June 15-Sept 28 in Horhizotrons in a greenhouse. Treatments began 14 days after planting (DAP). HRL increased linearly for the substrate water daily and the substrate rewatered once dried to 15% and increased quadratically in the substrate rewatered once dried to 10% moisture and the unwatered substrate (*P*<0.0001). (B) Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *I. floridanum* grown from March 15-July 3 in Horhizotrons in a greenhouse. Treatments began 65 days after planting (DAP). HRL increased linearly for the substrate water daily and the substrate rewatered once dried to 15% and increased quadratically in the substrate rewatered once dried to 10% moisture and the unwatered substrate (*P*<0.0001). Points are means of 50 observations.

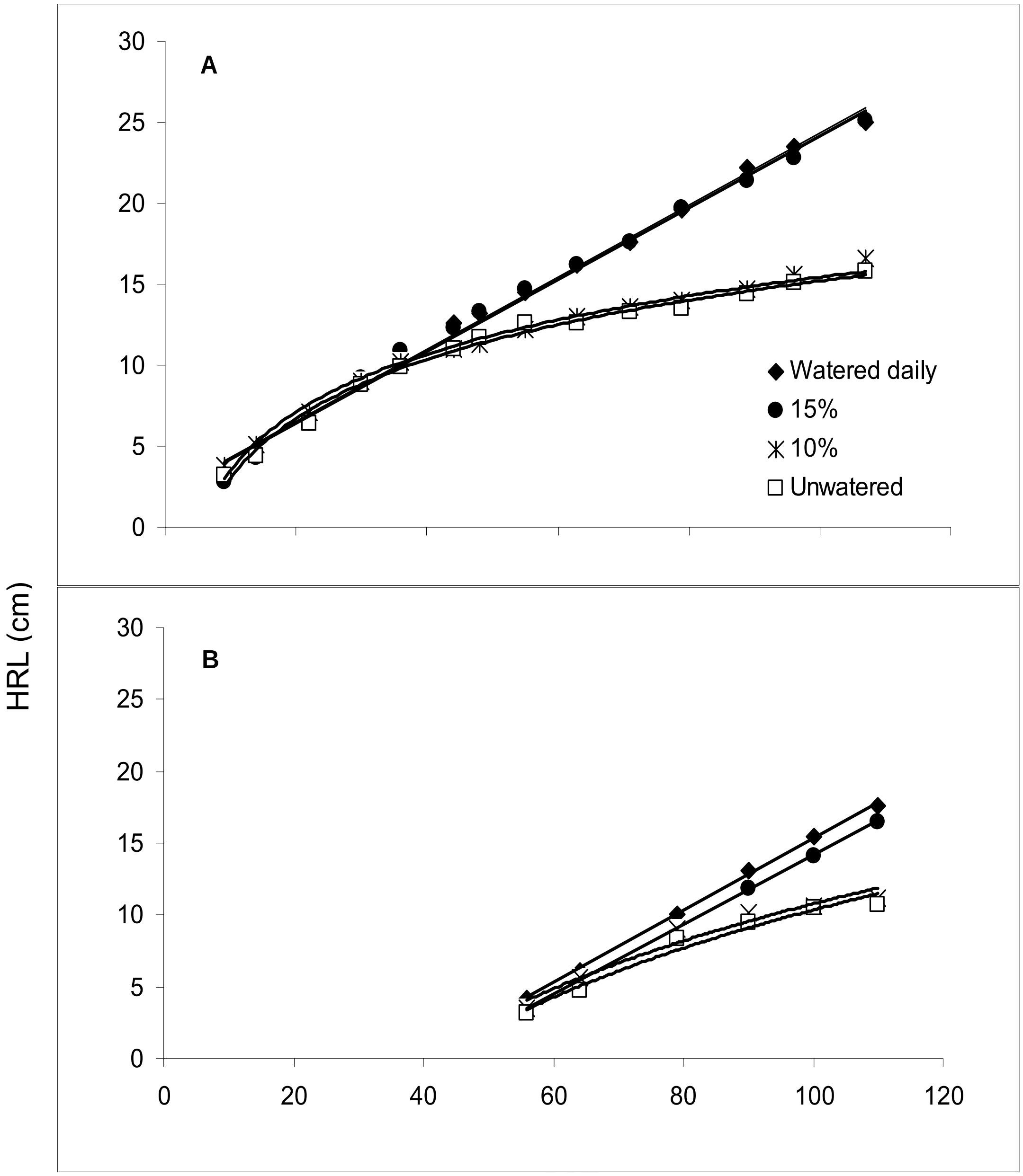


Figure 3. Effect of irrigation frequency on root dry weight of *M. cerifera* grown (A) from February 8 – April 28 in Horhizotrons in a greenhouse. Treatment began 14 days after planting (DAP). Treatments included: substrate watered (400mL) daily (~20% moisture), substrate rewatered (400mL) once dried to 15%, substrate rewatered (400mL) once dried to10% moisture, and substrate that remained unwatered throughout the study. (B) Effect of irrigation frequency on root dry weight of *M. cerifera* grown from February 8 – April 28 in Horhizotrons in a greenhouse. Treatment began 14 days after planting (DAP). Treatments included: substrate watered (400mL) daily (~20% moisture), substrate rewatered (400mL) once dried to 15%, substrate rewatered (400mL) once dried to10% moisture, and substrate that remained unwatered throughout the study. Means separated using LSD ($P < 0.05$). Means followed by the same letter are not significantly different ($P < 0.1124$).

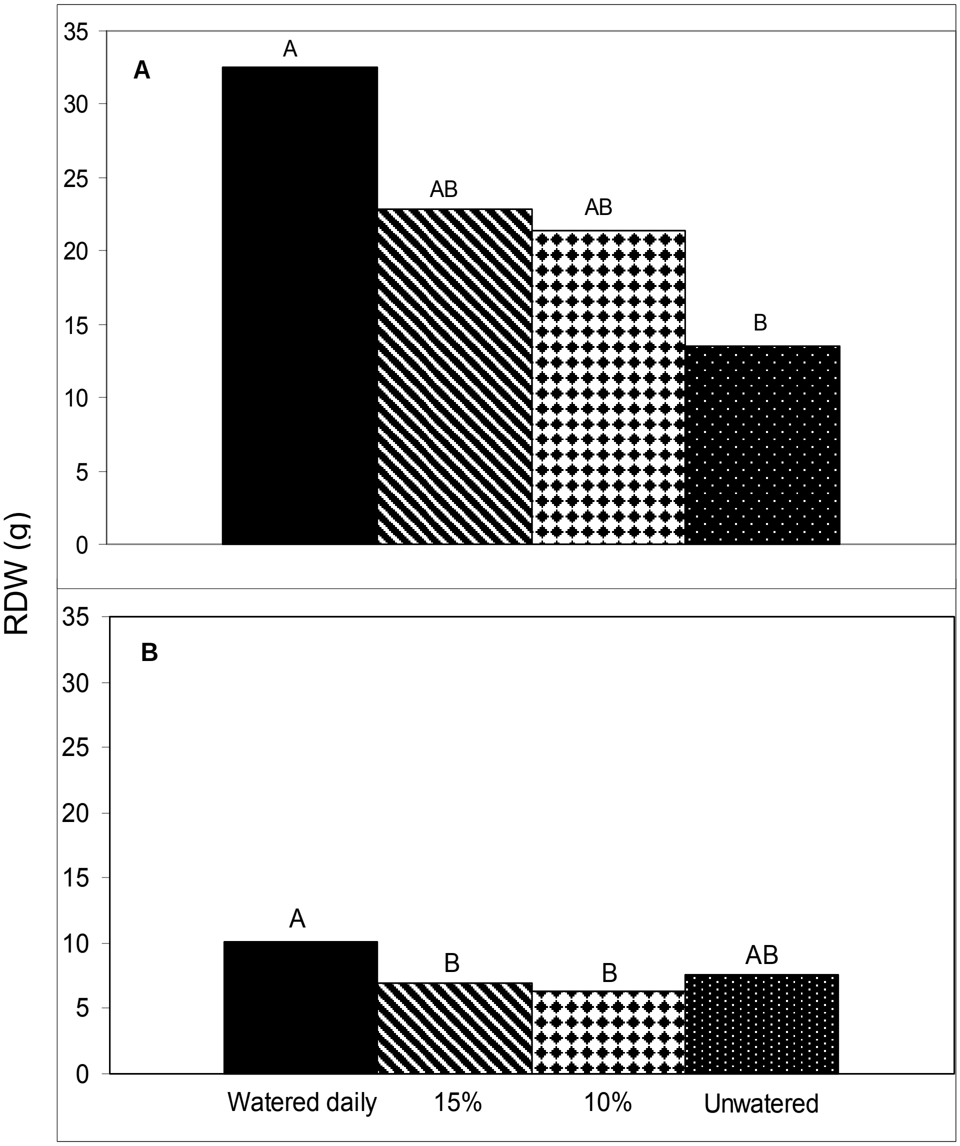
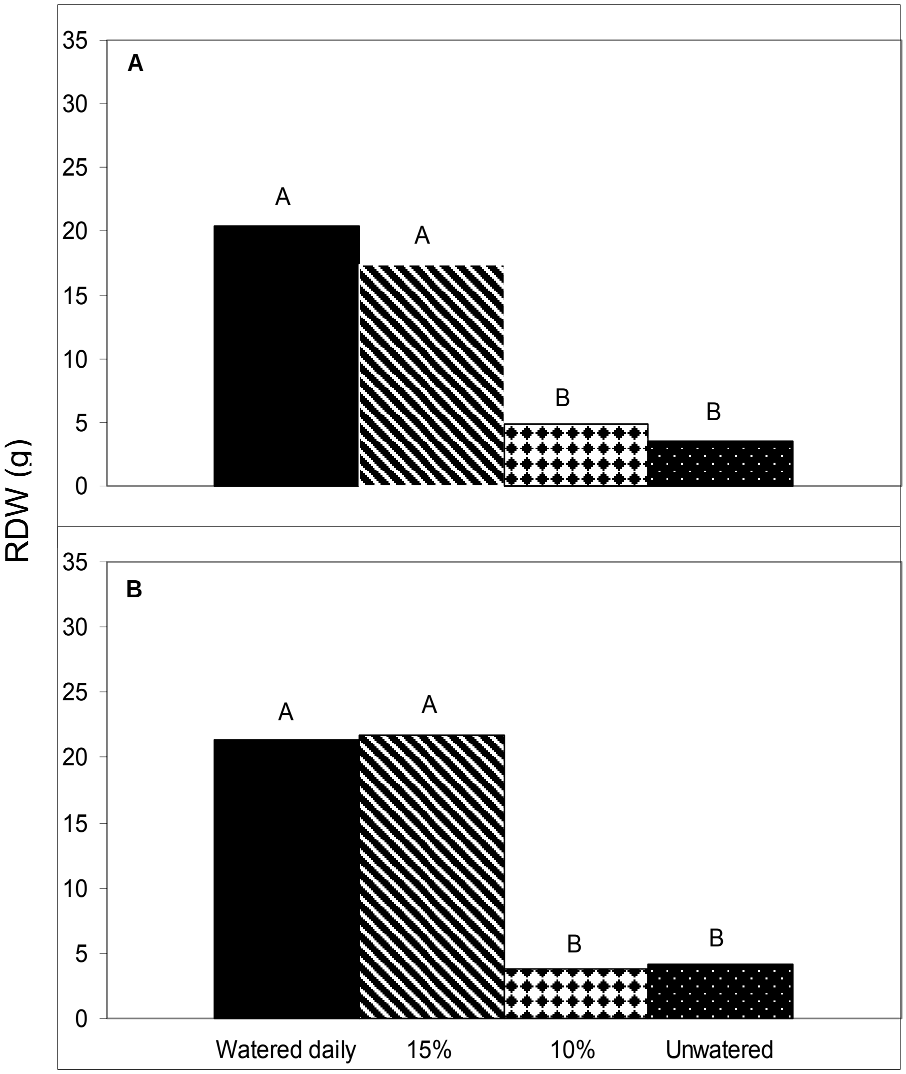


Figure 4. Effect of irrigation frequency on root dry weight of *I. floridanum* grown from (A) June 15-Sept 28 in Horhizotrons in a greenhouse. Treatment began 14 days after planting (DAP). Treatments included: substrate watered (400mL) daily (~20% moisture), substrate rewatered (400mL) once dried to 15%, substrate rewatered (400mL) once dried to10% moisture, and substrate that remained unwatered throughout the study. (B) Effect of irrigation frequency on root dry weight of *I. floridanum* grown from March 15-July 3 in Horhizotrons in a greenhouse. Treatment began 65 days after planting (DAP). Treatments included: substrate watered (600mL) daily (~20% moisture), substrate rewatered (600mL) once dried to 15%, substrate rewatered (600mL) once dried to10% moisture, and substrate that remained unwatered throughout the study. Means separated using LSD ($P < 0.05$). Means followed by the same letter are not significantly different ($P < 0.0001$).



CHAPTER 3

THE EFFECT OF IRRIGATION FREQUENCY ON POST-TRANSPLANT ROOT GROWTH, STEM WATER POTENTIAL, AND NET PHOTOSYNTHESIS OF *KALMIA LATIFOLIA*

Abstract.

Knowledge of plant responses to soil moisture deficit can provide information concerning relative drought tolerance and irrigation requirements of native landscape shrub species. Plants of *Kalmia latifolia* ‘Olympic Wedding’ (mountain laurel) were planted into Horhizotrons on 21 Nov. 2006. Each Horhizotron contained eight glass panes arranged to form four quadrants extending away from the original container root ball. In the first experiment one, of three different irrigation frequencies was assigned to all four quadrants of a Horhizotron, which consisted of watered-daily or rewatered once substrate percent moisture reached 15% or 10% by volume. Root balls of all plants received 600 mL water every two days. Stem water potential and net photosynthesis were measured for the control or once each substrate reached the desired minimum percent moisture. In a second experiment, four different irrigation frequencies were randomly assigned among the four quadrants in each Horhizotron and consisted of: watered-daily, unwatered, and rewatered once substrate percent moisture reached 15% or 10% moisture by volume. In both experiments, biweekly horizontal root length (HRL) was recorded by measuring the five longest roots (measured parallel to the ground) visible on each glass pane of a quadrant, and root dry weight (RDW), shoot dry weight (SDW), final growth indices (GI), and root and shoot visual quality ratings were determined upon completion of the study. During the first experiment, HRL was highest in the watered-daily substrate, however, HRL was highest in the unwatered substrate during the second experiment. There were no differences in RDW in either experiment. SDW and GI were highest for plants in the watered-daily substrate in the first experiment. Stem water potential increased with decreasing soil moisture, and net photosynthesis decreased with decreasing soil moisture. These experiments indicate that soil water deficit affects root growth of *K. latifolia* differently depending on if (A) the entire root ball is experiencing soil water deficit, or (B) if only part of the root ball is experiencing soil water deficit. Additionally, stem water potential and net photosynthesis provided good information about the response of *K. latifolia* to different irrigation frequencies.

Introduction

Water stress is often associated with poor appearance, dieback, or even death of transplanted container grown woody landscape plants (Beeson, 1994; Costello and Paul, 1975). Before being transplanted into the landscape, plants are usually in an environment of daily irrigation, but after transplanting into the landscape, irrigation may become far less frequent. Decrease in irrigation frequency along with the surrounding landscape soil drawing water away from the root ball can lead to severe water deficits within the root ball. Consequently, it has been suggested that irrigation should be increased from what the plant received in production until root growth extends away from the original container root ball into the surrounding landscape soil (Costello and Paul, 1975).

With many climate change scenarios suggesting an increase in aridity in many areas around the world, it is of considerable interest to investigate plant responses to water stress (Chaves et al., 2003). On a worldwide basis, drought (soil and/or atmospheric water deficit) poses an important environmental constraint to overall plant survival and productivity (Boyer et al., 1982). Therefore, it is necessary to have a better comprehension of the effect drought can have on plants. A better understanding could then facilitate improvement in management practices and breeding efforts in horticulture to adjust to climate change (Chaves et al., 2003).

The use of native plant species in the landscape could be one strategy to combat increasing drought. The appreciation of native plant species in the landscape has risen in recent years. The belief that plants (of a certain area) are more adapted to live in that environment and should replace landscape species that have high water requirements is becoming more popular (Meyer, 2005). With water restrictions being imposed by municipalities and increases in aridity, it is important to put into practice the use of drought tolerant plants and to evaluate the actual drought tolerance of these plants (Alder and Ostler, 1989). If these plants are used in the landscape, water usage by the homeowner and municipalities could be greatly reduced.

Plant productivity and overall survival of land plants is dependent upon having a healthy functioning root system (Wraith and Wright, 1998). Of the factors affecting post-transplant establishment in the landscape, root growth following transplanting is one of the most important (Watson and Himelick, 1982). In order for plants to receive the crucial elements needed for survival, particularly water and mineral nutrients, root growth following transplanting must occur.

Quantifying post-transplant lateral root growth away from the original root ball into surrounding landscape soil is important since plant establishment has been correlated with time to new root growth (Woods, 1959). Observation of post-transplant root growth into multiple rhizosphere conditions can be indicative of plant preference to rhizosphere conditions. In order to quantify root growth over time, several different techniques have been developed, including rhizotrons, minirhizotrons, and root dyes.

A new technique, the Horhizotron™, provides an inexpensive, versatile, and easy method of quantifying lateral root growth over time (Wright and Wright, 2004). The method is not destructive and provides a simple method for measuring lateral root growth from the original container root ball into a variety of rhizosphere conditions imposed upon a single plant.

In general, water potential of most land plants decreases with decreasing soil moisture (Boyer, 1969). During a drought event, net photosynthesis also usually decreases (Chaves, 2003). The decrease can be due to a decrease in light interception since leaf expansion is reduced and increases in leaf senescence. Another explanation for the decrease in photosynthesis is a decline in carbon fixation caused by a reduction in stomatal aperature.

Kalmia latifolia L. (mountain laurel) is a native broad-leaved evergreen shrub capable of growing up to 15 m. *K. latifolia* is considered one of the most prized native shrubs for landscaping (Dirr, 1998). *K. latifolia* can be planted in mass near shady borders, in full sun, or in rocky soils. Most notable about this species is the floral display. The flowers range from white to almost purple. Even though *K. latifolia* is prized by many plant enthusiasts, it frequently does not survive transplant into the landscape. The westward portion of its geographic distribution extends to Ohio, and the east part extends from Quebec to Florida. Work was conducted to compare the species drought tolerance with that of *Ilex crenata* Thunb. ‘Compacta’ (‘Compacta” Japanese holly), and found that *K. latifolia*’s poor performance in the landscape may be due to its relatively low drought tolerance and its slow rate of root growth (Wright et al, 2004; Wright et al., 2003). Based on results of these previous studies the objective of this study was to evaluate root growth and physiological responses of *K. latifolia* to three different irrigation frequencies.

Materials and Methods

Experiment 1. On 21 Nov. 2006, 20 plants of *K. latifolia* were removed from 19 L containers, and each plant was placed in the center of a single Horhizotron (Wright and Wright, 2004). Horhizotrons were placed on a greenhouse bench at the Patterson Horticulture Greenhouse Complex, Auburn University, and Auburn, Ala. (day/night temperatures set at 26/21°C). Each Horhizotron had four wedge-shaped quadrants, with each quadrant being constructed from two glass panes measuring 26 cm x 20 cm in height, extending away from the root ball of each plant. Quadrants were filled with Greens Grade™ Profile™ (Profile Products, Buffalo Grove, Ill.), a calcined clay material, that had been amended with 9.5 $kg \cdot yd^{-3}$ $17N\text{-}5P_2O_5\text{-}11K_2O$ (Pursell Industries, Sylacauga, Ala.), 1.2 $kg \cdot m^{-3}$ dolomitic limestone, and 0.9 $kg \cdot m^{-3}$ Micromax™ (The Scotts Co., Marysville, Ohio). Greens Grade Profile was chosen because its chemical and physical properties are more similar to field soil than sand and is easily removed from roots to determine root dry weight, upon completion of the study. Substrate in all quadrants in each Horhizotron received daily irrigation with 600mL tap water until roots had grown approximately 5 cm into each quadrant at which time treatments were initiated. Original plant root balls received 600mL tap water daily throughout the experiment. On 8 Mar. 2007 (108 days after planting, DAP) treatments were initiated and included three different irrigation frequencies, and a single treatment was applied to all four quadrants of a Horhizotron. Treatments included: substrate watered daily (~20% moisture by volume), substrate rewatered with 600mL tap water once dried to 15% moisture by volume, and substrate rewatered with 600mL tap water once dried to 10% moisture by volume. This volume of tap water was required at each rewatering to fully wet the substrate. Substrate percent moisture (by volume) was measured every other day at 0800 HR using a Theta probe (Delta-T Devices Ltd., Cambridge, England). Percent moisture within a treatment was determined by randomly measuring two quadrants within each Horhizotron in each treatment.

When substrate reached the desired minimum percent moisture for that treatment, stem water potential (Ψ_{stem}) was determined using a pressure chamber (PMS Instruments, Corvallis, Ore.) and the following methodology (Scholander et al., 1965). At 1000 HR, a single, recently mature leaf, located near the apex of a shoot, was selected on each of the five plants within a treatment and wrapped first with plastic wrap, then wrapped with aluminum foil. At 1300 HR the leaf was removed with a razor blade at the base of the petiole, placed into a cool dark portable cooler and taken to a lab for immediate determination of Ψ_{stem} (Turner and Begg, 1973). On the same days Ψ_{stem} measurements were taken, net photosynthesis was measured using an open infrared gas exchange analyzer (IRGA) system (Li-6400) (Li-Cor Inc., Lincoln, Ne.) at 10 sequential light intensities, 2200, 2000, 1800, 1500, 1200, 1000, 800, 500, 200, and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, beginning with the highest light intensity. One light curve was taken for each of the 5 plants within a treatment on one recently matured leaf, located near the apex of a shoot. Net photosynthesis was measured between 1000 HR and 1300 HR. One reading was taken for each PAR level on each leaf. A leaf area of 1 cm^2 was used in the cuvette of the Li 6400.

Rewatering of a treatment occurred at 1300 HR, immediately following completion of all measurements, with water applied to the original container root ball and quadrants. The day after rewatering, the Ψ_{stem} and net photosynthesis measurements were conducted in the same manner as described above.

Stem water potential and net photosynthesis were measured for the watered daily control on 161, 170, and 182 DAP. Based on the length of time it took the substrate in each treatment to reach its target percent moisture, Ψ_{stem} was measured 161, 170, and 182 DAP in the substrate rewatered at 15% moisture and 156 and 168 DAP in the substrate rewatered at 10% moisture. Net photosynthesis was measured on the same days as Ψ_{stem}.

On 17 Apr. (148 DAP) Ψ_{stem} was measured on one recently matured leaf located near the shoot apex from each of five watered-daily control plants every two hours for 24 hours starting at 0500 HR with 30 minutes between leaf wrapping (described above) and measurement (Turner and Begg, 1973).

Once every two weeks, horizontal root length (measured parallel to ground, HRL) of the five longest roots visible along each glass pane that formed each side of a quadrant (two glass panes per quadrant). Upon completion of the experiment (30 Mar. 192 DAP) and once dead shoots had been removed, growth indices (GI) were calculated for all plants, by measuring the plant height (h), widest width (w_1), and width perpendicular to the widest width (w_2) and using the formula GI = $(h + w_1 + w_2)/3$. Ratings of shoot and root visual quality were also recorded upon completion of the study (Table 1). At the end of the study, roots that had grown into each quadrant were cut from the original root ball, and the substrate was gently rinsed from those roots. The roots were then dried for 48 hours at 66 °C, and dry weights were recorded. In addition to root dry weights (RDW), shoot dry weights (SDW) were also recorded by removing living shoots from the original container root ball and drying for 120 hours at 66 °C.

This experiment was a randomized complete block design, with five blocks per treatment and four Horhizotrons (one per treatment) comprising one block. Data were analyzed using GLM procedures and regression analysis, and means were separated using Least Significant Difference ($P<0.05$) (SAS Institute Inc., 2003).

Experiment 2. On the same plating date as described above, 5 *K. latifolia* plants were removed from 19 L containers, and each plant was placed in the center of five separate Horhizotrons . On 8 Mar. 2007 (108 days after planting, DAP) treatments were initiated and included four different irrigation frequencies randomly assigned to the four quadrants within each Horhizotron. Treatments were four different irrigation frequencies. Treatments included: substrate watered daily (~20% moisture by volume), substrate rewatered once dried to 15% moisture, substrate rewatered once dried to 10% moisture, and substrate that remained unwatered throughout the experiment. Biweekly root growth measurements were taken by measuring the horizontal root length (measured parallel to ground, HRL) of the five longest roots visible along the glass panes that formed each side of a quadrant (two glass panes per quadrant). At the end of the study (205 DAP), roots that had grown into each quadrant were cut from the original root ball, and the substrate was gently rinsed from those roots. These roots were then dried for 48 hours at 66 °C, and RDW were recorded. SDW was also recorded. Initial and final plant growth indices were also measured. Ψ_{stem} measurements were also taken 163, 183, 189, and 196 DAP in the same matter as described above.

The experimental design was a randomized complete block design, with five blocks and one Horhizotron equaling one block. Data were analyzed using General Linear Model procedures and regression analysis, and means were separated using Least Significant Difference ($P<0.05$) (SAS, 2004).

Results

Experiment 1. Horizontal root length (HRL) increased cubically throughout the experiment (Fig. 1; Table 2) in all treatments. From the beginning of the experiment to the end, HRL in the watered-daily control and in the substrate rewatered at 15% moisture were higher than HRL in the substrate rewatered at 10% moisture with the HRL in the watered-daily control and HRL in the substrate rewatered at 15% moisture being similar at the end of the experiment ($P < 0.0001$) (Fig. 1, Table 2). There were no differences between treatments for RDW ($P < 0.4206$), but SDW was highest ($P < 0.0002$) in the watered-daily control and the substrate rewatered at 15% moisture (Table 3). Plants in the watered-daily control had the highest value for GI with GI in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture being similar and lower ($P < 0.0039$) (Table 3). Root quality (RQ) and shoot quality (SQ) were highest in the watered-daily control ($P < 0.0001$) (Table 1).

Stem water potential of the watered-daily control plants was highest at 0130 HR and lowest at 1130 HR (Fig. 2). Stem water potential for the watered-daily control did not differ throughout the experiment. Stem water potential was significantly affected by irrigation frequency and whether Ψ_{stem} was measured before rewatering or 24 hours after rewatering, and the interaction between irrigation frequency and rewatering was also significant for the first and second dry-down cycles (Table 4). During the third dry-down cycle there were no differences in Ψ_{stem} between irrigation frequencies, but there were differences in Ψ_{stem} before and after rewatering (Table 4). Overall, plants in the watered-daily control and in the substrate rewatered at 15% moisture had higher Ψ_{stem} than in the substrate rewatered at 10% moisture (Fig. 3 B, C). Stem water potential for plants in the substrate rewatered at 15% moisture differed between before and after rewatering until dry-down cycle 5, at which point before and after rewatering Ψ_{stem} values were not different (Table 3B). Values for Ψ_{stem} before rewatering for plants in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture increased over the course of the experiment (Fig. 3; Table 4), and values for Ψ_{stem} after rewatering stayed about the same or decreased slightly (Fig. 3, Table 4).

In the watered-daily control, net photosynthesis changed with change in PAR (Fig. 4). Figure 4 is shown as a representative graph for the watered-daily control. Rewatering had a significant effect on net photosynthesis for both the first and second dry-down cycles (Table 5). Within irrigation frequency, net photosynthesis was different among dry-down cycles for plants in the substrate rewatered at 15% moisture, but not in the substrate rewatered at 10% moisture (Table 5). The interaction between the dry-down cycle and whether or not net photosynthesis was measured before or after rewatering was significant in the substrate rewatered at 15% moisture and not in the substrate rewatered at 10% moisture (Table 6). The interaction between irrigation frequency and whether or not net photosynthesis was measured before or after rewatering was significant in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture (Table 7). Regression analysis of change in net photosynthesis with change in PAR was performed separately within each dry-down cycle for before rewatering and 24 hours after rewatering (Table 5). For the first two dry-down cycles in the substrate rewatered at 15% moisture the trend was different before and after rewatering (Fig. 5 A, B, Table 5). However, on the third dry-down cycle the equations and responses are similar (Fig. 5 C, Table 5). Regression equations for the substrate rewatered at 10% moisture were different before and after rewatering but did not change over time (Fig. 6 A, B, Table 5,).

Net photosynthesis at 1800 PAR was compared among irrigation frequencies averaged across rewatering, and separately before and 24 hours after rewatering. Plants in the watered-daily control had the highest rate of net photosynthesis at 1800 PAR followed by the substrate rewatered at 15% moisture and lastly the substrate rewatered at 10% moisture (Table 8). This same result was seen before rewatering but was different after rewatering with net photosynthesis in the watered-daily control and in the substrate rewatered at 15% moisture being similar and higher than in the substrate rewatered at 10% moisture (Table 8).

Experiment 2. HRL increased quadratically in the watered-daily control substrate, but increased cubically in the substrate rewatered at 15% moisture, in the substrate rewatered at 10% moisture, and in the substrate that remained unwatered (Table 9, Fig. 7). At the beginning of the experiment, HRL in the substrate rewatered at 10% moisture and the unwatered substrate were higher than HRL in the watered-daily control substrate and in the substrate rewatered at 15% moisture, and continued this trend continued until the end of the experiment (178 DAP) (Fig. 7). By the end of the experiment HRL in the watered-daily control substrate was lowest ($P<0.0004$), however, there were no differences in root dry weight (RDW) between any of the treatments ($P < 0.1124$) (Fig. 8).

Average shoot dry weight (SDW) was 0.39 kg and average growth index (GI) was 70.3 (data not shown). Stem water potential increased initially during the experiment, but decreased towards the end of the experiment (Fig. 9)

Discussion

Experiment 1. More HRL in the watered-daily control and in the substrate rewatered at 15% moisture than in the substrate rewatered at 10% moisture agrees with other results for plant response to moisture stress, since root growth in inversely proportional to water stress (Becker et al., 1987). HRL in all treatments increased over time initially, but prolonged exposure to soil water deficit halted that in root length (Fig. 1). This is a well-documented occurrence that many plants exhibit when exposed to decreased soil moisture (Wraith and Wright, 1998). Lower HRL in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture may be due to a reduction in cell elongation which can be attributed to low turgor pressure at low soil water potentials (Sharp et al., 1988). Even though the final HRL in the substrate rewatered at 10% moisture was lowest, RDW showed no differences between any of the treatments. This could be due in part to changes in root diameter. In order to combat increased mechanical impedance of the substrate, caused by a decrease in substrate percent moisture, roots may have been growing thicker in order to penetrate the substrate (Spollen et al., 2000; Davies and Bacon, 2003).

SDW and GI were highest in the watered-daily control. Lower SDW, GI, and visual quality in the substrate rewatered at 15% moisture and the substrate rewatered at 10% moisture were most likely due to the decrease in net photosynthesis (Fig 5 and 6), which resulted in decreased carbon assimilation (Chaves, 1991). Stomata for the plants rewatered at 15% moisture and 10% moisture closed in response to moisture stress (Chaves, 2003). Once stomata close in response to low soil and plant water potential (Fig. 5 and 6), carbon dioxide may not be available in sufficient amounts to satisfy the photosynthesis machinery of a plant, resulting in a decrease in carbon assimilation (Yordanov et al. 2000). Stomatal closure may result signaling from shoot dehydration (Chaves, 1991), while other studies suggest signaling due to root dehydration is responsible (Davies and Bacon 2003).

Stem water potential for the watered-daily control was similar throughout the experiment. Stem water potential for plants rewatered at 15% moisture was lower before than after rewatering, but this difference became less significant as the experiment continued. This might suggest that plants were possibly adjusting initially to the prolonged water deficit. One way plants can adjust is osmotically accumulating alcohols, carbohydrates, or proteins inside cell walls (Yordanov et al., 2000). Plants may also close stomata to conserve water and increase water use efficiency (Chaves, 2003). Stem water potential for the substrate rewatered at 10% moisture had large fluctuations between before and after rewatering, with values for Ψ_{stem} before rewatering increasing, suggesting the plants may have been acclimating to soil water deficit.

Net photosynthesis for plants in the watered-daily control did not differ over time. Net photosynthesis for plants in the substrate rewatered at 15% moisture was higher following rewatering during the first two dry-down cycles, but this difference became less significant as the experiment progressed. By the end of the experiment, net photosynthesis of plants in the substrate rewatered at 15% moisture were similar to that of plants in the substrate rewatered at 10% moisture. This result suggests that duration of the drought in the substrate rewatered at 15% moisture had about the same effect as the severity of the drought in the substrate rewatered at 10% moisture.

Other species native to the southeast typically have photosynthesis measurements of around 12 $\mu mol•CO_2•m^{-2}•s^{-1}$ eastern redbud (*Cercis Canadensis* L.) (Griffin et al., 2004), 18 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ Florida anise tree (*Illicium floridanum* Ellis) (personal observation), and 19 $\mu mol•CO_2•m^{-2}•s^{-1}$ wax myrtle [*Morella cerifera* (L.) Small] (personal observation). The peak measurement for the control in this experiment was around 5 $\mu mol•CO_2•m^{-2}•s^{-1}$, so to decrease what small amount of carbon assimilation this plant is capable of could definitely make this plant difficult to transplant (Chaves, 1991). Lower measurements of net photosynthesis and subsequent low levels of carbon assimilation, for plants in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture may have caused lower HRL, RDW, SDW, GI, and visual quality when compared to measurements in the watered-daily control. Water deficit lowered net photosynthesis measurements and also may have played a role in less cell elongation for roots and shoots. This experiment shows that Ψ_{stem} and net photosynthesis are good indicators of the physiological effect irrigation frequency has on *K. latifolia.*

Experiment 2. Root growth occurred in all treatments, but the most HRL occurred in the unwatered substrate. This result may indicate that *K. latifolia* may have been distributing water (within the root system) from areas of higher moisture content to the areas where moisture was limiting, a process known as hydraulic redistribution (Coutts, 1982; Warren et al., 2007). Applying water to part of a root system equalizes root xylem potential, and longitudinal resistance to water is frequently small (Slavikova, 1967), thus allowing relatively unimpeded movement of water across the root system. The transfer of water within the root system is probably due to a water potential gradient which is formed, and is thought to enable plants to continue growth, maintain turgor, and absorb nutrients necessary for development (Breazeale, 1930). Even though there was more HRL in the unwatered substrate, RDW showed no differences among treatments. This result might suggest roots in the unwatered substrate were smaller in diameter than roots in the watered-daily control, a result which has been observed in other plant species (Davies and Bacon, 2003). Sharp et al. (1988) suggests plants with this root pattern are able to allocate limited carbohydrate supply to root length rather than diameter in order to explore a larger soil volume.

Conclusion. When comparing the results between experiment 1 and experiment 2, the effect of irrigation frequency on HRL was different. HRL in experiment 1 was highest in the watered-daily substrate and in the substrate rewatered at 15% moisture, while in experiment 2 HRL was highest in the substrate that remained unwatered. It appears that irrigation frequency affects *K. latifolia* differently depending on whether the entire rhizosphere is receiving irrigation, or if only part of the rhizosphere is receiving irrigation. The results from both experiments suggest that *K. latifolia* root growth will continue in all directions similarly if the rhizosphere is homogenous, but has the capability to hydraulically redistribute water from areas of higher moisture content to areas where moisture is limiting. Average GI and SDA were lowered some in experiment 2, but was still closest to that of the watered-daily control in experiment 1. This might suggest that even though plants in experiment 2 were receiving less water, having ample water every two days in at least one of the quadrants is enough to not limit carbon assimilation, thereby allowing shoot growth to continue.

When placing *K. latifolia* in the landscape, it would be beneficial for the entire root ball to receive irrigation at least every two days to not have a negative effect on post-transplant root growth. However, *K. latifolia* may receive irrigation to only part of the root ball, at least every two days, and have almost no effect on shoot growth or quality. In either scenario, the slow rate of root growth experienced by *K. latifolia* is going to greatly reduce the chance of finding other water resources in the rhizosphere.

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Table 1. Scale used to visually rate quality of roots and shoots and the effect of irrigation frequency on root and shoot quality of *K. latifolia* 178 days after planting (DAP) in Horhizotrons in a greenhouse in Auburn, Ala.

Ratings	Root Quality (RQ)[z]	Ratings	Shoot Quality(SQ)[y]
0	No roots visible	0	No living shoots
1	Almost no roots visible	1	Some dead shoots
2	Sparse proliferation	2	Yellow and dropping foliage
3	Intermdiate proliferation	3	Some yellow and wilted foliage
4	Somewhat dense proliferation	4	Some yellow foliage
5	Very dense proliferation	5	No visible symptoms

Irrigation Frequency	RQ	SQ
Watered-daily	4.4 a	4.6 a
15%[x]	4.0 b	3.4 b
10%[w]	3.8 b	2.6 c

[z] Based on averge root density and length

[y] Based on leaf color, canopy density, and wilting

[x] Rewatered once substrate percent moisture reached 15% by volume

[w] Rewatered once substrate percent moisture reached 10% by volume

Table 2. Effect of irrigation frequency on final horizontal root length (HRL[z]) of *K. latifolia* growing in Horhizotrons in Auburn, Ala. (178 DAP[y]), regression equations for change in HRL over time with corresponding R^2 term and significance of regression equation (P-value), and significance of treatment main effects and interactions for HRL. Plants were grown in a greenhouse (21 Nov. 2006 – 17 May 2007).

Irrigation Frequency	HRL (cm)	Equation[x]	R^2	P-value
Watered-Daily	6.50 a[w]	$y = -0.000017x^3 + 0.00691x^2 - 0.92x + 34.85$	0.24	< 0.0001
15%[v]	6.51 a	$y = -0.000013x^3 + 0.00454x^2 - 0.49x + 18.68$	0.12	< 0.0001
10%[u]	5.64 b	$y = -0.000002x^3 + 0.00003x^2 + 0.14x - 7.090$	0.21	< 0.0001
Significance	P-value			
Irrigation frequency	0.4110			
DAP	< 0.0001			
Irrigation frequency * DAP	0.0003			

[z] HRL = root length measured parallel to the ground

[y] DAP = days after planting

[x] y = HRL, x = DAP

[w] Lowercase letters denote mean separation (n = 50) among treatments by LSD $P < 0.05$ (SAS Inc., 2003)

[v] Rewatered once substrate percent moisture reached 15% by volume

[u] Rewatered once substrate percent moisture reached 10% by volume

Table 3. The effect of irrigation frequency on root dry weight (RDW), shoot dry weight (SDW), and growth indices (GI) of *K. latifolia* growing in Auburn, Ala. 178 days after planting (DAP). Plants were grown in a greenhouse (21 Nov. 2006 – 17 May 2007).

Irrigation Frequency	RDW (g)	SDW (kg)	G.I.[z]
Watered-daily	0.39 a[y]	0.42 a	72.5 a
15%[x]	0.85 a	0.38 ab	67.4 b
10%[w]	0.59 a	0.34 b	63.1 b

[z] Growth index = (height + widest width + perpendicular width)/3

[y] Lowercase letters denote mean separation (n = 5) among treatments by LSD at $P < 0.05$ (SAS Inc., 2003)

[x] Rewatered once substrate percent moisture reached 15% by volume

[w] Rewatered once substrate percent moisture reached 10% by volume

Table 4. Effect of irrigation frequency and rewatering on stem water potential (Ψ_{stem}) of *K. latifolia* in substrate rewatered at 15% moisture or substrate rewatered at 10% moisture and significance of irrigation frequency and rewatering main effects and interactions for Ψ_{stem} *of K. latifolia*. Plants were grown in a greenhouse in Auburn, Ala. (21 Nov. 2006 – 17 May 2007).

15%[z]				10%[y]		
Dry-down cycle		Ψ_{stem} (MPa) Before	Ψ_{stem} (MPa) After	Dry-down cycle	Ψ_{stem} (Mpa) Before	Ψ_{stem} (Mpa) After
1	D[x]	-1.36 b[w]	-0.58 a	1	-2.32 b	-0.38 a
2	C	-0.91 b	-0.66 a	2	-2.01 b	-1.04 a
3	C	-1.06 b	-0.62 a	3	-1.23 b	-0.61 a
4	B	-0.85 b	-0.50 a			
5	B	-0.88 b	-0.71 b			
6	A	-0.63 b	-0.72 b			

	P-value Dry-down cycle 1	P-value Dry-down cycle 2	P-value Dry-down cycle 3
Irrigation frequency	0.0069	0.0002	0.3662
Rewatering[v]	< 0.0001	0.0008	0.0014
Irrigation frequency*rewatering	0.0006	0.0154	0.5137

[z] Rewatered once substrate percent moisture reached 15% by volume

[y] Rewatered once substrate percent moisture reached 10% by volume

[x] Upper case letters denote mean separation (n = 5) for before values between dry-down cycles by LSD P < 0.05 (SAS Inc., 2003

[w] Lower case letters denote mean separation (n = 5) within each dry-down cycle by LSD P < 0.05 (SAS Inc., 2003)

[v] Before rewatering or 24 hours after rewatering

Table 5. Regression equations for net photosynthesis (Ps) with PAR as affected by irrigation frequency, rewatering, and dry-down cycle with corresponding R^2 and significance of equation (P-value).

Watered-daily control	Equation[y]	R^2	P-value
N/A	$y = -0.000003532x^2 + .0123x - 7.04$	0.63	0.0003

Rewatered at 15% moisture by volume[x]

Rewatering	Dry-down cycle[z]	Equation	R^2	P-value
Before[w]	1	$y =-0.0000022x^2 + 0.0061x - 3.50$	0.36	0.0003
After	1	$y = -0.0000018x^2 + 0.0067x - 4.71$	0.57	0.0037
Before	2	$y = -0.0000025x^2 + 0.0061x - 5.31$	0.35	< 0.0001
After	2	$y= -0.0000018x^2 + 0.0053x - 3.28$	0.36	0.0026
Before	3	$y = -0.0000028x^2 + 0.0053x - 5.03$	0.48	< 0.0001
After	3	$y = -0.0000027x^2 + 0.0062x - 5.73$	0.13	0.0112

Rewatered at 10% moisture by volume[v]

Rewatering	Dry-down cycle	Equation	R^2	P-value
Before	1	$y = -0.0000013x^2 + 0.0031x - 2.76$	0.36	< 0.0001
After	1	$y = -0.0000016x^2 + 0.0062x - 5.20$	0.59	0.0073
Before	2	$y = -0.0000013x^2 + 0.0026x - 2.24$	0.14	0.0085
After	2	$y = -0.0000016x^2 + 0.0059x - 4.43$	0.68	0.0004

[z] Period between rewatering

[y] y = Ps, x = PAR

xRewatered once substrate percent moisture reached 15% by volume

[w] Before rewatering or 24 hours after rewatering

[v] Rewatered once substrate percent moisture reached 15% by volume

[u] P-values determined using GLM (SAS Inc., 2003)

Table 6. Significance of main effects and interactions for block, irrigation frequency[z], PAR[y], and rewatering[x] for net photosynthesis within the first two dry-down cycles[w].

	P-value	
	Dry-down cycle	
Significance	1	2
Irrigation frequency	0.0049	0.0104
PAR	< 0.0001	< 0.0001
Irrigation frequency*PAR	0.0064	0.9416
Rewatering	0.0548	<0 .0001
Rewatering*PAR	0.0043	< 0.0001
Irrigation frequency*rewatering	0.6713	0.0009
Irrigation frequency*rewatering*PAR	0.8232	0.2173

[z] Rewatered once substrate percent moisture reached 15% by volume or once substrate percent moisture reached 10% by volume

[y] Photosythetically active radiation (μmol•m^{-2}•s^{-1})

[x] Before an irrigation event or 24 hours after an irrigation event

[w] Period between rewatering

vP-values determined using GLM (SAS Inc., 2003)

Table 7. Significance of interactions and main effects of dry-down cycle[x], PAR[w], and rewatering[v] for net photosynthesis within each irrigation frequency.

	P-value	
	Irrigation frequency	
Significance	15%[z]	10%[y]
Dry-down cycle	0.0019	0.3438
PAR	< 0.0001	< 0.0001
Dry-down cycle*PAR	0.0011	0.9773
Rewatering	< 0.0001	< 0.0001
Rewatering*PAR	0.0163	< 0.0001
Rewatering*dry-down cycle	< 0.0001	0.9699
Rewatering*dry-down cycle*PAR	0.9999	0.9984

[z] Rewatered once substrate percent moisture reached 15% by volume

[y] Rewatered once substrate percent moisture reached 10% by volume

[x] Period between rewatering

[w] Photosynthically active radiation ($\mu mol \bullet m^{-2} \bullet s^{-1}$)

[v] Before rewatering or 24 hours after rewatering

[u] P-values determined using GLM (SAS Inc., 2003)

Table 8. Net photosynthesis (Ps) at 1800[z] for plants in the watered daily control substrate, the substrate rewatered at 15% moisture by volume, and the substrate rewatered at 10% moisture by volume averaged over rewatering (A), before rewatering (B), and after rewatering (24 hours) (C).

	Net Ps (μmol CO_2•m^{-2}•s^{-1})		
	A	B	C
Irrigation frequency		Before[y]	After[y]
Watered-Daily	2.49 a[x]	2.45 a	2.45 a
15%[w]	-0.41 b	-1.48 b	0.66ab
10%[v]	-1.95 c	-2.72 c	-1.16 c
P-value	< 0.0001	< 0.0001	0.0128

[z] Photosynthetically active radiation (μmol•m^{-2}•s^{-1})

[y] Before rewatering or 24 hours after rewatering

[x] Lowercase letters denote mean separation (n = 5) among treatments winin column by LSD P<0.05 (SAS Inc., 2003)

[w] Rewatered once substrate percent moisture reached 15% by volume

[v] Rewatered once substrate percent moisture reached 10% by volume

Table 9. Effect of irrigation frequency on final horizontal root length (HRL[z]) of *K. latifolia* growing in Horhizotrons in Auburn, Ala. (206 DAP[y]), regression equations for change in HRL over time with corresponding R^2 term and significance of regression equation (P-value), and significance of treatment main effects and interactions for HRL. Plants were grown in a greenhouse (21 Nov. 2006 – 5 June 2007).

Treatment	HRL (cm)	Equation[x]	R^2	P-value
Watered-Daily	3.44 b[w]	$y = 0.0010x^2 - 0.33x - 22.46$	0.09	< 0.0001
15%[v]	3.62 ab	$y = -0.00014x^3 + 0.07x^2 + 10.09x - 510.52$	0.20	< 0.0001
10%[u]	3.94 ab	$y = -0.00006x^3 - 0.28x^2 + 4.336x - 219.12$	0.10	0.0123
Unwatered	4.06 b	$y = 0.00007x^3 - 0.03x^2 + 5.16x - 264.12$	0.13	0.0113
Significance	P-value			
Treatment	0.6565			
DAP	0.0004			
Treatment x DAP	0.0035			

[z] HRL = root length measured parallel to the ground

[y] DAP = days after planting

[x] y = HRL, x = DAP

[w] Lowercase letters denote mean separation (n = 50) among treatments by LSD at $P < 0.05$ (SAS Inc., 2003)

[v] Rewatered once substrate percent moisture reached 15% by volume

[u] Rewatered once substrate percent moisture reached 10% by volume

Figure 1. Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *K. latifolia* grown from 21 Nov. 2006 – 29 May 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007, 108 days after planting (DAP). HRL increased cubically throughout the study for the watered-daily control, the substrate rewatered once dried to 15% moisture, and the substrate rewatered once dried to 10% moisture (P < 0.0001).

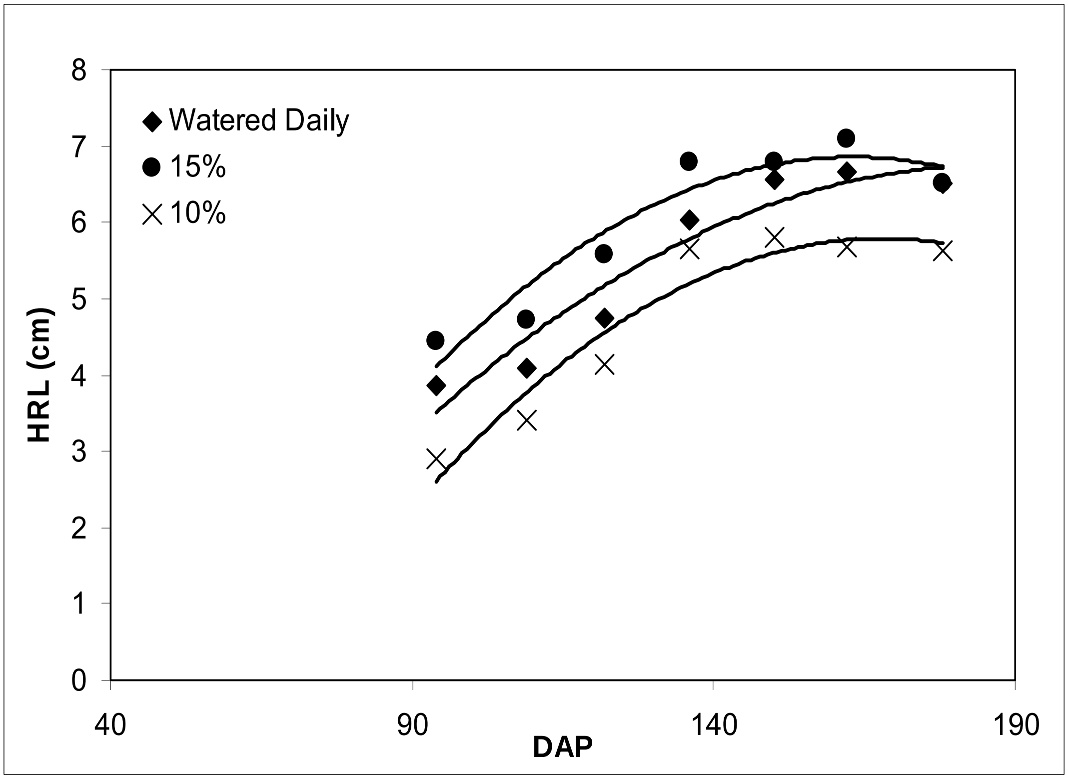


Figure 2. Stem water potential Ψ_{stem} of watered-daily control plants. Measurements were taken every two hours starting at 0500 HR 17 Apr. 2007 and ending at 0500 HR 18 Apr. 2007.

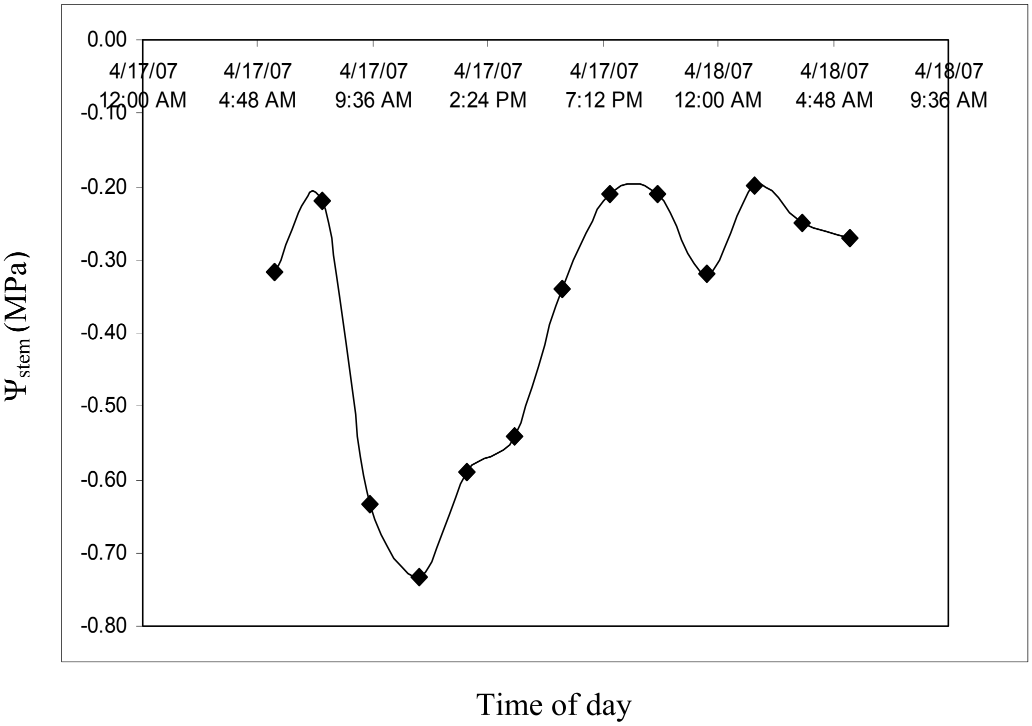


Figure 3. Effect of irrigation frequency on Ψ_{stem} of *K. latifolia* grown from 21 Nov. 2006 – 29 May 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007, 108 days after planting (DAP). Irrigation frequency included watered-daily control (A), rewatered when substrate percent moisture reached15% moisture by volume (B), and rewatered when substrate percent reached 10% moisture by volume (C).

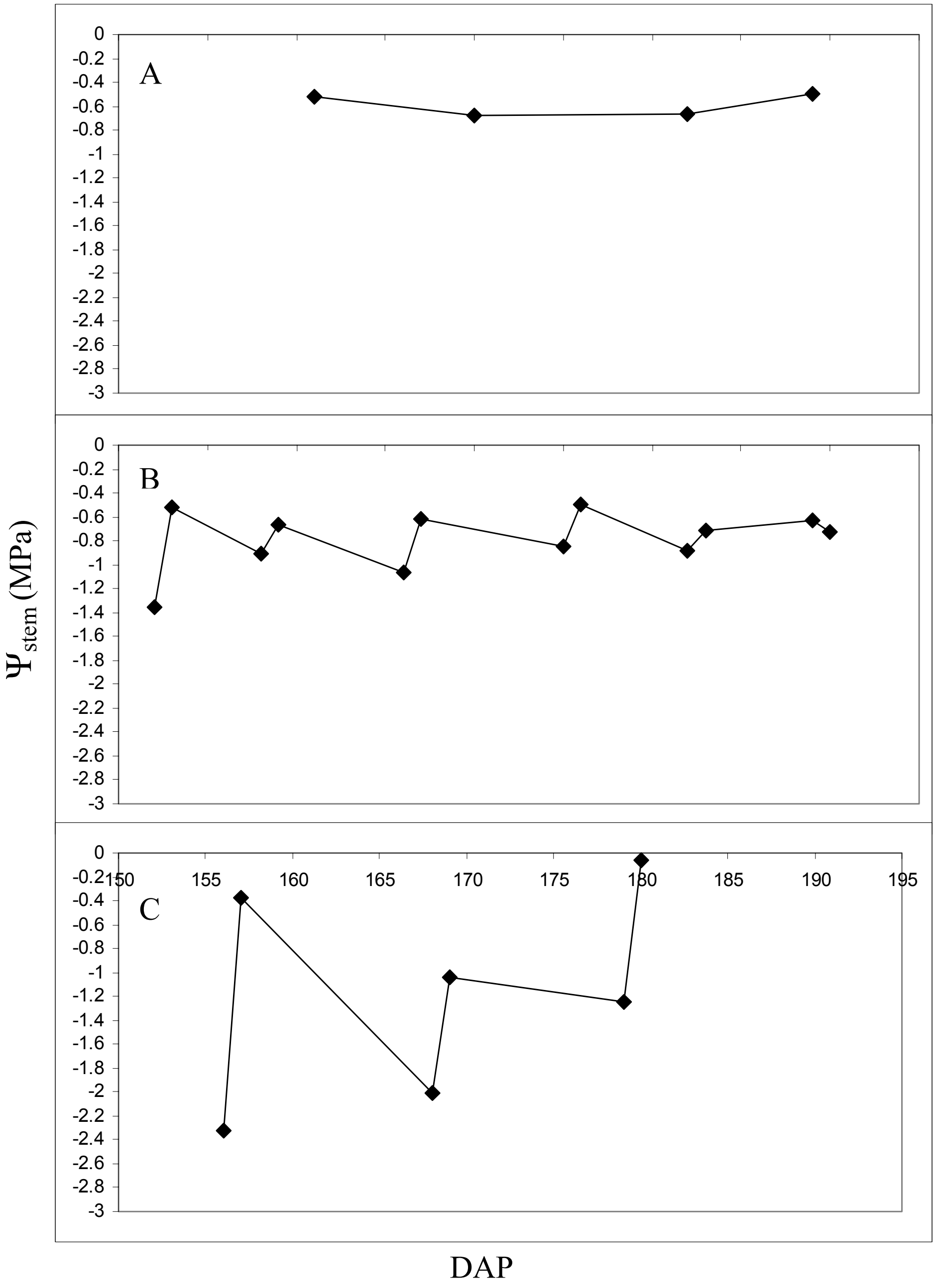


Figure 4. Change in net photosynthesis (Ps) with change in photosynthetically active radiation (PAR) of *K. latifolia,* watered-daily and grown from 21 Nov. 2006 – 29 May 2007, 170 days after planting (DAP), in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007.

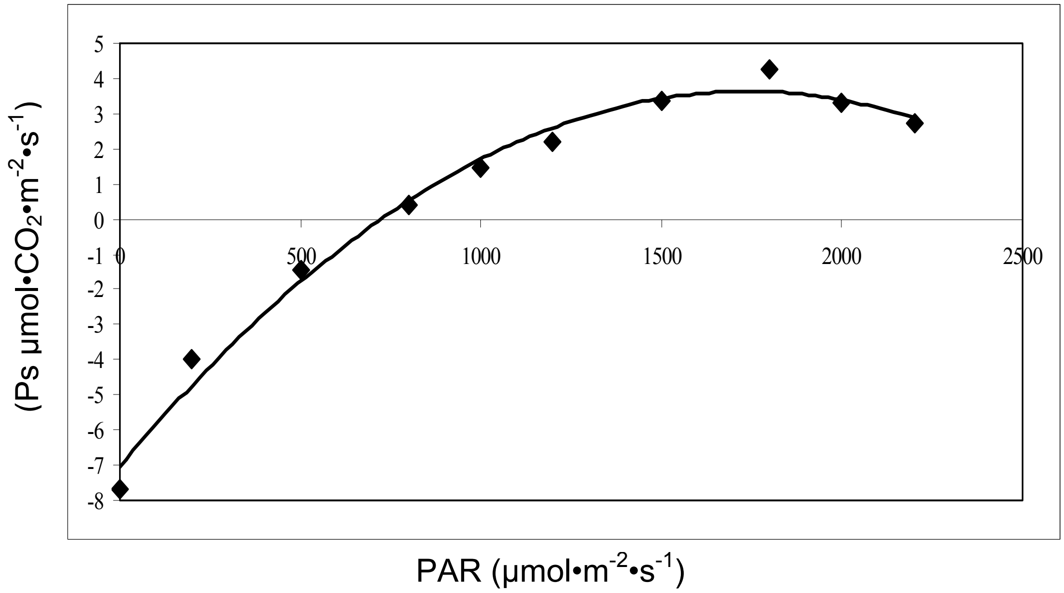


Figure 5. Change in net photosynthesis (Ps) with change in photosynthically active radiation (PAR) of *K. latifolia* during dry-down cycle 1 (A), dry-down cycle 2 (B), dry-down cycle 3 (C), ,rewatered at 15% moisture and grown from 21 Nov. 2006 – 29 May 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007. Measured before rewatering or 24 hours after rewatering.

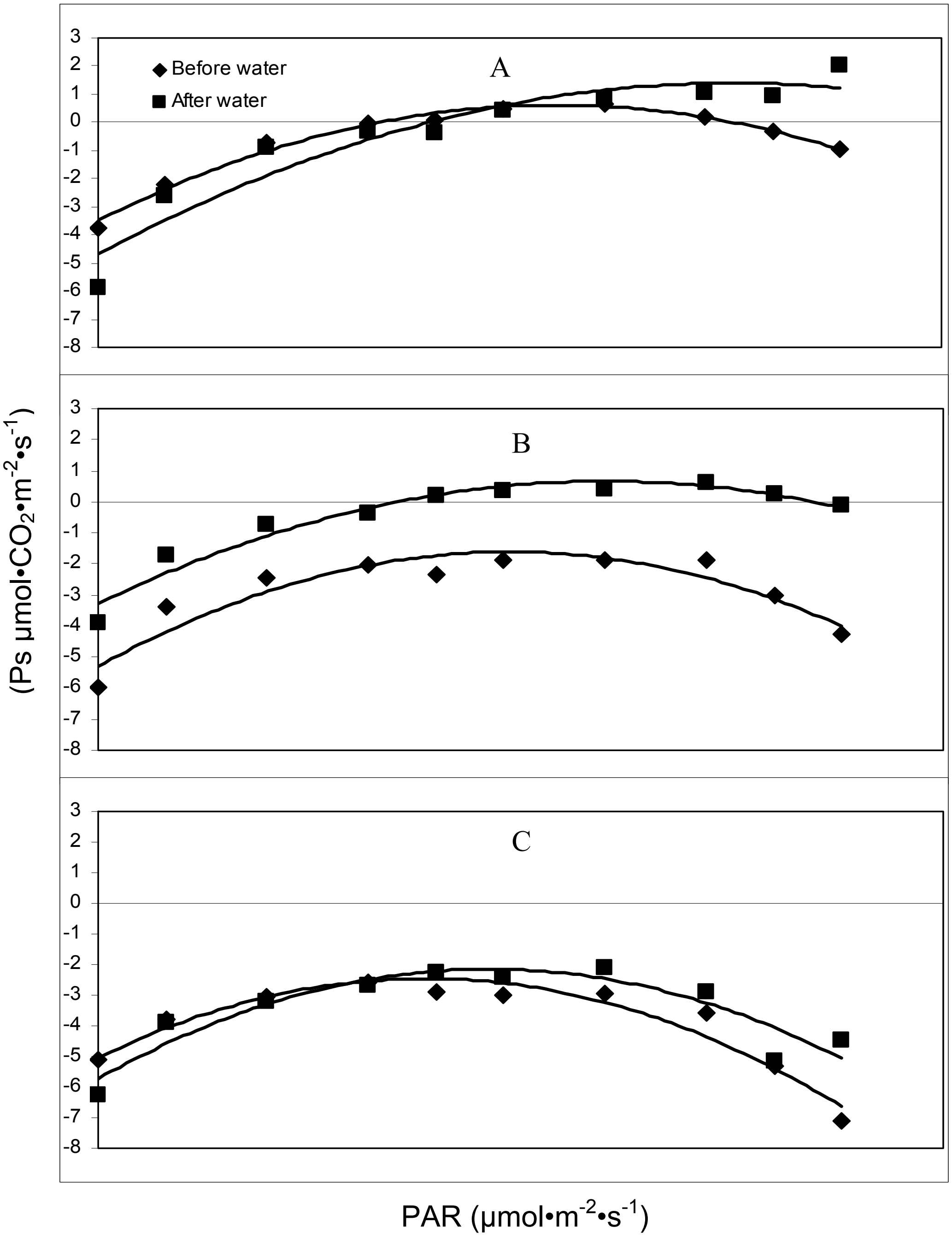


Figure 6. Change in net photosynthesis (Ps) with change in photosynthically active radiation (PAR) of *K. latifolia* during dry-down cycle 1 (A), and dry-down cycle 2 (B), rewatered at 10% moisture and grown from 21 Nov. 2006 – 29 May 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007. Measured before rewatering or 24 hours after rewatering.

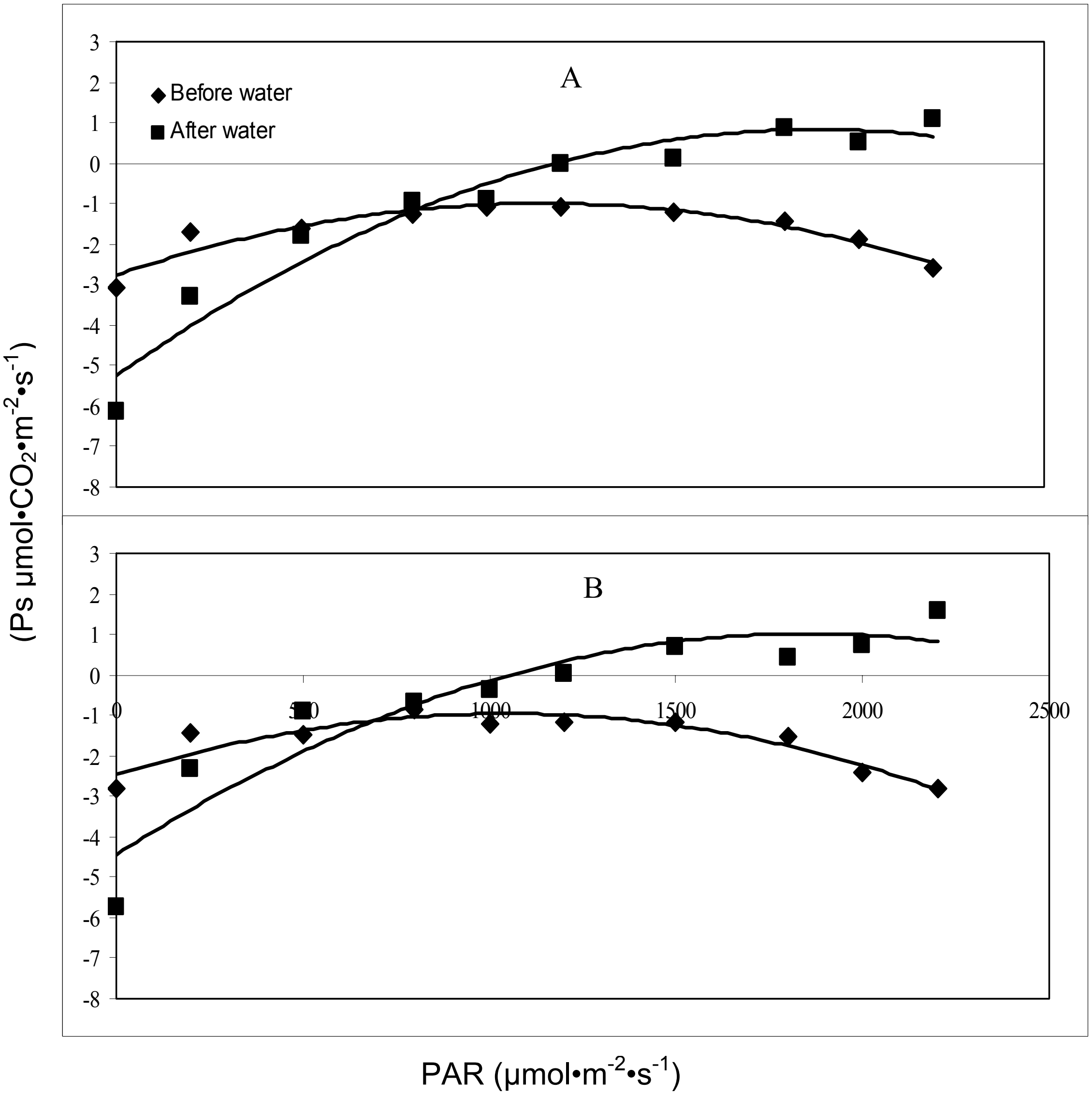


Figure 7. Effect of irrigation frequency on horizontal root length (measured parallel to the ground, HRL) of *K. latifolia* grown from 21 Nov. 2006 – 5 June 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Treatments began 8 Mar. 2007, 108 days after planting (DAP). HRL increased quadratically throughout the study for the watered-daily control, and cubically for the substrate rewatered once dried to 15% moisture, and the substrate rewatered once dried to 10% moisture (P < 0.0001).

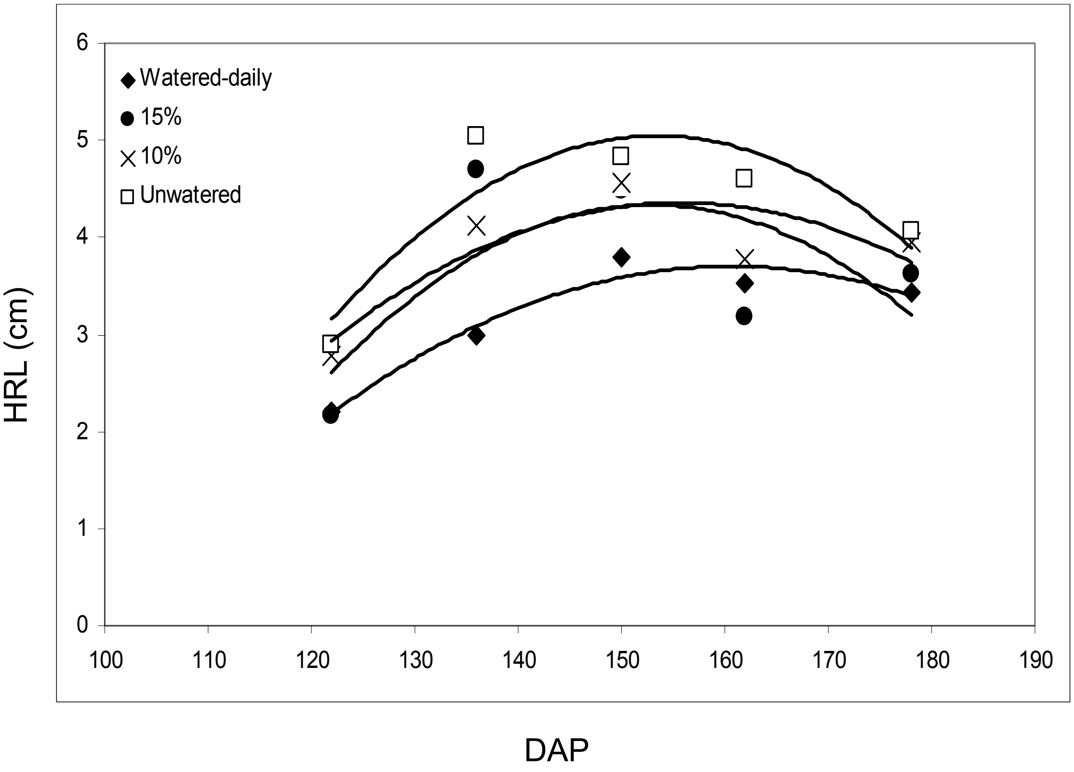


Figure 8. Effect of irrigation frequency on root dry weight of *K. latifolia* grown (A) from 21 Nov. 2006 – 5 June 2007 in Horhizotrons in a greenhouse. Treatment began 8 Mar. 2007 108 days after planting (DAP). Treatments included: substrate watered (600mL) daily (~20% moisture), substrate rewatered (400mL) once dried to 15%, substrate rewatered (400mL) once dried to10% moisture, and substrate that remained unwatered throughout the study. Means separated using LSD (*P* < 0.05). Means followed by the same letter are not significantly different (*P* < 0.1124).

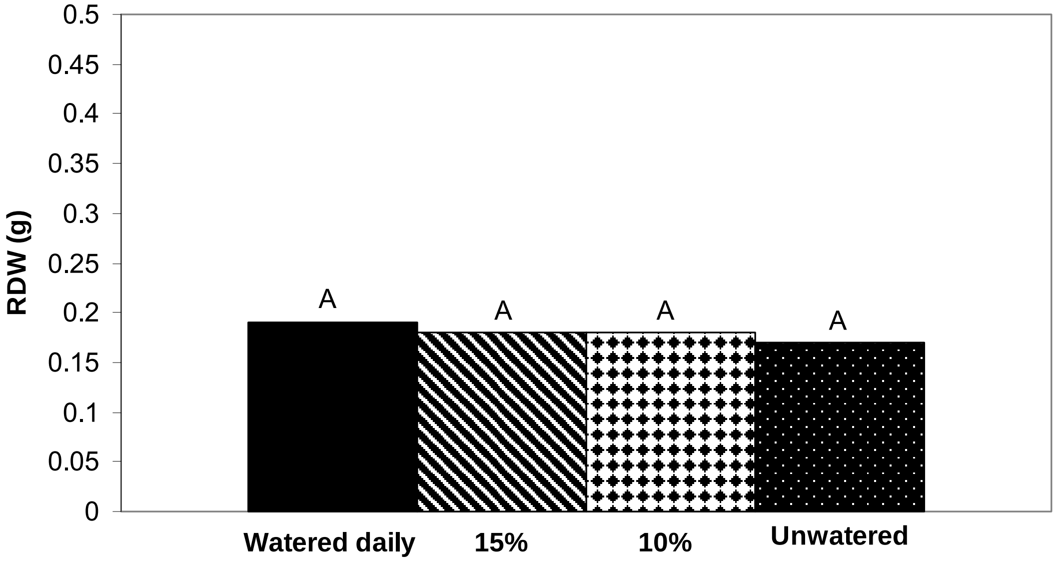
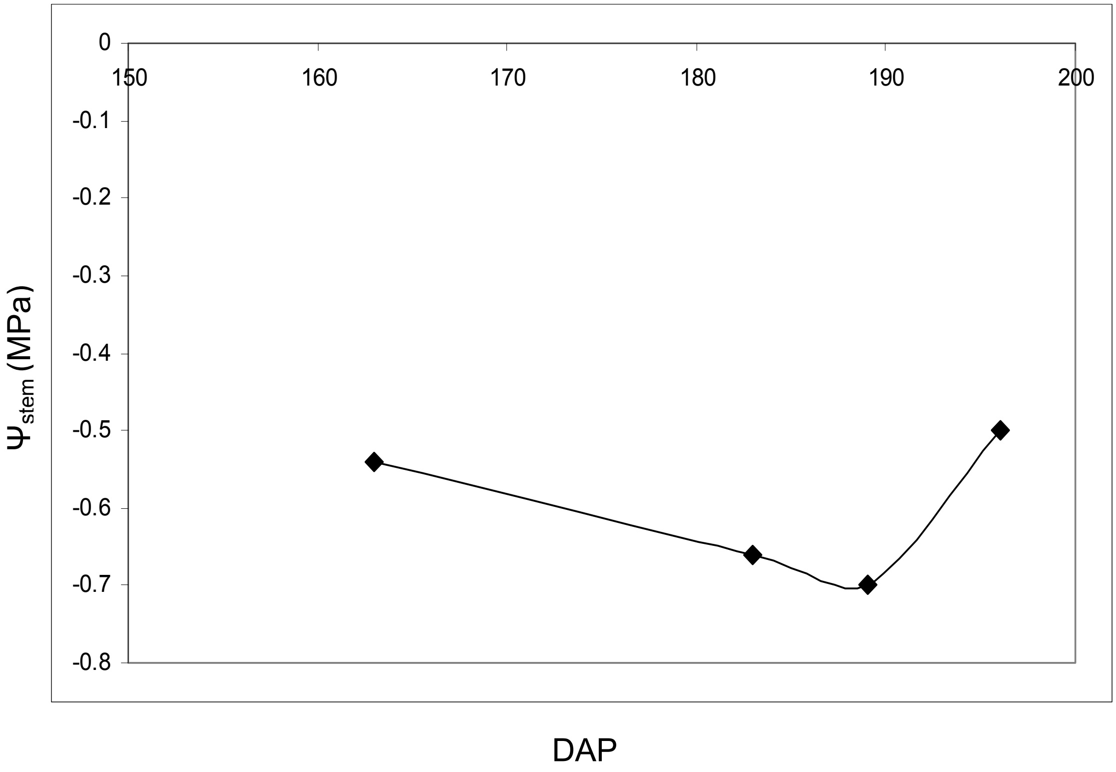


Figure 9. Stem water potential of *K. latifolia* grown from 21 Nov. 2006 – 5 June 2007 in Horhizotrons in a greenhouse in Auburn, Ala. Four treatments were applied to a single Horhizotron and consisted of a watered-daily control, rewatered once substrate percent moisture reached 15% by volume, rewatered once substrate percent moisture reached 10% by volume, and a substrate that remained unwatered throughout the experiment. Treatments began 8 Mar. 2007, 108 days after planting (DAP).



CHAPTER FOUR

FINAL DISCUSSION

The Effect of Irrigation Frequency on Post-transplant Root Growth of *Morella cerifera* and *Illicium floridanum.*

Morella cerifera (L.) Small and *Illicium floridanum* Ellis are both shrubs native to the southeast and used often in the landscape. *M. cerifera* is known to grow in a variety of harsh environments, such as growing in slightly brackish waters, bogs, upland woods, and old fields, where as *I. floridanum* is typically found in moist to almost inundated areas. Since these two plant species have such differing native habitats, we wanted to evaluate the effect of irrigation frequency on horizontal root growth. During this experiment plants of both *Morella cerifera* and *Illicium floridanum* were placed in Horhizotrons™ and were exposed to four different irrigation frequencies consisting of: watered-daily control, unwatered, and watered once 10% or 15% moisture by volume was reached.

Results from this experiment suggest *M. cerifera* can continue sustained levels root growth in all directions as long as some portion of the root ball is receiving some water. *I. floridanum* displayed the same result, but at a much lesser degree. This is probably due to each plant's native habitat. Probably, as each plant evolved, they developed features that allowed them to thrive in their respective habitat, for example, *M. cerifera,* native to drier, upland areas, may have developed the ability to hydraulically redistribute water within its own root system to maintain turgor to the roots growing in drier portions of the rhizosphere, thereby allowing the plant to discover untapped water resources. However, *I. floridanum* did not display the same magnitude of root growth as *M. cerifera*, suggesting the plant, native to more moist areas, never experienced this adaptation. After all, why *I. floridanum* need to hydraulically redistribute water within its root system if it was growing in a swamp?

This experiment showed a great contrast between two plants of differing native habitats when exposed to differing levels of soil moisture especially since the southeast is experiencing a prolonged drought. If this study was repeated, it would be nice to compare root growth of native shrubs, when exposed to differing levels of soil moisture, to that of a shrubs from a different area. It would also be nice to conduct a study of this nature in the field. If this was achieved, we could formulate a definitive answer as to whether native plants perform better in the landscape as a plant considered to be invasive. This, in turn, could lead to more eco-friendly landscapes.

The effect of irrigation frequency on post-transplant root growth, stem water potential, and net photosynthesis of *K. latifolia*

Kalmia latifolia is a plant known to be difficult to transplant into the landscape. Studies in the past have focused on this problem, but none have looked at the effect of soil moisture deficit on physiology of *K. latifolia*. Since *K.*

latifolia is such a prized plant in the landscape, it is considerable interest to evaluate this plant, especially since the southeast is experiencing a prolonged drought.

During experiment one, plants were placed in Horhizotrons and were exposed to three different irrigation frequencies, and a single treatment was applied to all four quadrants of a Horhizotron. Treatments included: substrate watered daily (~20% moisture by volume), substrate rewatered with 600mL tap water once dried to 15% moisture by volume, and substrate rewatered with 600mL tap water once dried to 10% moisture by volume. During experiment two, treatments included four different irrigation frequencies randomly assigned to the four quadrants within each Horhizotron. Treatments were four different irrigation frequencies. Treatments included: substrate watered daily (~20% moisture by volume), substrate rewatered once dried to 15% moisture, substrate rewatered once dried to 10% moisture, and substrate that remained unwatered throughout the experiment.

Results for horizontal root length (HRL) were different depending on whether the rhizosphere experience the single irrigation frequency per Horhizotron or four different irrigation frequencies per Horhizotron. It was interesting to see the contrast between the two experiments because during experiment one, HRL was highest in the watered-daily control, but for experiment 2, HRL was highest in the unwatered substrate. I think this plant most defiantly displays hydraulic redistribution properties if only part of the root ball is receiving irrigation. This is most likely a foraging strategy the plant has developed over time to find other resources in the rhizosphere. In experiment one, however, HRL was greatest in the watered-daily substrate, suggesting the plant was receiving adequate irrigation and grew root in all directions. Both experiments did not show any differences in root dry weight. Shoot dry weight, shoot quality, and growth indices were all greatest in the watered-daily substrate. There was no decline in net photosynthesis or decrease in stem water potential (Ψ_{stem}) in experiment one the watered-daily control. Irrigation frequency decreased net photosynthesis and decreased Ψ_{stem} in the substrate rewatered at 15% moisture and in the substrate rewatered at 10% moisture. The low values for net photosynthesis in either of the drier irrigation frequencies could be explained by *K. latifolia* being a drought avoider. In order to survive, the plant was closing stomata and in essence, shutting down to conserve water. A feature that probably helps the plant survive in its native habitat in the mountains.