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The Relationship Between Salinity and Drought Tolerance In Turfgrasses and Woody Species

Nisa Leksungnoen
Utah State University

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THE RELATIONSHIP BETWEEN SALINITY AND DROUGHT TOLERANCE
IN TURFGRASSES AND WOODY SPECIES

by

Nisa Leksungnoen

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Plant Science

Approved:

____________________  _________________________
Roger K. Kjelgren    Grant E. Cardon
Co-Major Professor   Committee Member

_________________________ _________________________
Paul G. Johnson       Thomas A. Monaco
Co-Major Professor   Committee Member

_________________________ _________________________
Richard C. Beeson, Jr.  Mark R. McLellan
Committee Member   Vice President for Research and
                  Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2012
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Both salinity and drought stresses induce osmotic stress. Thus, cross-tolerance responses and mechanisms may occur in plants. The overall objectives of this study were to determine morphological and physiological responses and mechanisms of turfgrasses and woody species under salinity and drought stress conditions, and determine the relationship between drought and salinity tolerance ability in those species.

Five turfgrass entries, ‘Gazelle’ and ‘Matador’ tall fescue (TF), ‘Midnight’ Kentucky bluegrass (KBG), PI368233 (Tolerant KBG), and PI372742 (Susceptible KBG), and three woody species, bigtooth maple (xeric-non saline), bigleaf maple (mesic-non saline) and Eucalyptus (mesic-saline) were compared.

For the drought study, water was withheld in Chapter 2 while the dry down treatment was based on daily evapotranspiration (ET) in Chapters 5 and 6. For the salinity study, NaCl and CaCl$_2$ in turfgrasses at electrical conductivity (EC) of 1, 6, 12,
18, and 30 dS m\(^{-1}\) (Chapter 3) and woody species at EC of 1, 3, 6, 9, and 12 dS m\(^{-1}\) (Chapter 4).

Susceptible KBG was sensitive to salinity but equally drought tolerant as other turfgrasses entries. Salinity tolerant turfgrasses could lower their water potential (\(\psi_{\text{leaf}}\)) and showed high K\(^+\):Na\(^+\). Under drought stress, above ground tissues of all entries went brown when soil water content was beyond permanent wilting point, indicating an equal response to drought.

In woody species, Eucalyptus maintained acceptable visual appearance under salinity stress while bigtooth maple maintained this under drought stress. Bigleaf maple was susceptible to both drought and salinity. Under salinity stress, bigleaf maple showed signs of leaf injury at 3 dS m\(^{-1}\) while bigtooth maple showed at 6 dS m\(^{-1}\) but leaf injury did not occur in Eucalyptus even at 12 dS m\(^{-1}\), due to an ability to exclude salts at root level. Under drought stress, Eucalyptus and bigleaf maple showed anisohydric behaviors in which water uptake was maintained. In contrast, bigtooth maple stomata closed in order to conserve water along lead to maintaining acceptable visual appearance over drought periods. However, bigtooth maple was not growing but surviving while Eucalyptus and bigleaf maple kept growing until no water was available and faced fatal injury.
PUBLIC ABSTRACT

The Relationship Between Drought and Salt Tolerance in Turfgrasses and Woody Species

Nisa Leksungnoen

Both salt and water deficit make it difficult for plants to uptake water from soil. Thus, plants under those conditions may respond and deal with them similarly. The overall objectives of this study were to 1) determine visual appearance and physiological responses, and mechanisms to deal with salt and water deficit of turfgrasses and woody species, and 2) determine the relationship between salt and water deficit tolerance ability in those species.

Five turfgrass entries, ‘Gazelle’ and ‘Matador’ tall fescue (TF), ‘Midnight’ Kentucky bluegrass (KBG), PI368233 (Tolerant KBG), and PI372742 (Susceptible KBG), and three woody species, bigtooth maple (xeric-non saline), bigleaf maple (mesic-non saline) and Eucalyptus (mesic-saline) were compared.

For the water deficit study, there was no irrigation in Chapter 2 while dry down treatment was based on daily water loss in Chapters 5 and 6. For the salinity study, NaCl and CaCl₂ were used in turfgrasses at salt levels of 1, 6, 12, 18, and 30 dS m⁻¹ (Chapter 3) and woody species at 1, 3, 6, 9, and 12 dS m⁻¹ (Chapter 4).

Susceptible KBG was sensitive to salts but equally tolerant under water deficit as other turfgrasses. Salt tolerant turfgrasses could extract more water from soil and did not absorb salts into their tissues, while Susceptible KBG absorbed salt ions and transported
to shoots, causing dead leaves. Under water deficit, leaves of all entries were dead at the same level of soil water content when there was no water for the plant to extract.

In woody species, Eucalyptus maintained acceptable visual appearance under salt stress while bigtooth maple showed this under water deficit. Bigleaf maple was sensitive to both drought and salinity. Eucalyptus had an ability to exclude salts at the roots which made it more tolerant to salt than bigtooth and bigleaf maple. Under water deficit, Eucalyptus and bigleaf maple maintained water uptake and grew normally until there was no water available to be extracted and they died. In contrast, bigtooth maple conserved water in tissues to maintain acceptable visual appearance but not growing over a drought period.
I would like to express my gratitude to the Ananthamahidol Foundation for giving me a chance to complete my Ph.D. It was my honor to be selected for this scholarship. I would like to thank my advisors, Dr. Roger Kjelgren and Dr. Paul Johnson, for their help and guidance through this process – especially Dr. Roger who suggested I apply to USU and helped me in every way to get into the program. I could not have done this without his support. I will be eternally indebted to committee members Dr. Grant Cardon, Dr. Richard Beeson, Jr., and Dr. Tom Monaco for their invaluable advice, critical reviews, and suggestions for improving my dissertation. Thanks to those who helped in other ways on this project, including Graham Hunter, Alec Hay, Jonathan Carlisle, and Austin Hawk.

But most of all, I give my sincere gratitude to my family in Thailand for their mental support. Even if we could not see each other I could definitely feel your love in my heart. I would also give heartfelt thanks to my extended family which I found here in Logan: Khun Roni, who took care of me when I first came here and has always been there for me when I needed lots of hugs, and Mom Ladda who has been always generously kind whenever I needed good food. Many thanks also go to all of my Thai friends for their help and support in all respects throughout the entire time.

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CHAPTER 1
INTRODUCTION

Abiotic stresses, including drought and salinity, occur naturally (Dai, 2011; Jacobsen and Adams, 1958). However, both stresses have been expanding due to human activities such as deforestations, salt mining (Ghassemi et al., 1995), poor irrigation water (Marcum, 2006), and emissions of greenhouse gases (IPCC, 2000). Due to climate changes, drought and salinity are predicted to be widespread all over the world (Dai, 2011) from which plants will encounter multifaceted challenges from stresses leading to reduction in growth and biomass (Ainsworth and Ort, 2010).

HOW SALINITY AND DROUGHT STRESSES ARE RELATED

Salinity and drought stress show a high degree of similarity with respect to physiological, biochemical, molecular and genetic effects (Sairam and Tyagi, 2004). Physiological drought occurs when soluble salt levels in the soil solution are high enough to limit water uptake due to low water potential, thereby inducing drought stress (Carrow and Duncan, 1998). The major difference between the low-water-potential environments caused by salinity versus drought is the total amount of water available. During drought, a finite amount of water can be obtained from the soil profile by the plant, causing ever-decreasing soil water potential. In most saline environments, a large amount of water is at a constant, but under low water potential. Plants have a chance to adjust their osmotic potential, which prevent loss of turgor and generate a lower water potential that allows plants to access water in the soil solution for growth (Taiz and Zeiger, 2006).
Both stresses lead to cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm into the intracellular space resulting in a reduction of the cytosolic and vacuolar volumes. Early responses to water and salt stress are largely identical except for the ionic component in the cells of plants under salt stress. These similarities include metabolic processes, e.g., a decrease of photosynthesis or increase in levels of the plant hormonal processes (ABA). High intracellular concentrations of sodium and chloride ions are an additional problem of salinity stress (Bartels and Sunkar, 2005).

Thus, plants may use common pathways and components in the stress response relationship known as cross-tolerance, which allows plants to acclimate to a range of different stresses after exposure to one specific stress (Pastori and Foyer, 2002). The common signals and elements are found as plants are exposed to salinity, cold, or drought stresses (Tuteja, 2007). Thus, a salinity tolerant species could also be drought tolerant or vice versa, and have similar mechanisms to cope with those stresses (Ashraf and O’Leary, 1996; Farooq and Azam, 2001; Glenn et al., 2009; Trivedi et al., 1991).

**SALINITY-AND DROUGHT-STRESSES EFFECTS ON PLANT GROWTH**

**Osmotic stress**

Osmotic stress, caused by both salinity and drought stress, induces turgor reduction; thus, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive. The smaller leaf area transpires less water, effectively conserving a limited water supply over a longer period (Taiz and Zeiger, 2006). When transpiration
decreases and leaf temperature becomes warmer than the air temperature, some of the extra energy in the leaf is dissipated as sensible heat loss. Small leaves like grasses tend to remain close to air temperature even when transpiration is greatly slowed because of their low boundary layer resistance. In contrast, large leaves in woody plants or dense canopy have larger boundary layer resistance and dissipate less thermal energy (per unit leaf area) by direct transfer of heat to the air which makes larger leaves higher temperature (Gate, 1968; Taiz and Zeiger, 2006). Under stress, plant growth declines due to stomatal closure, causing CO$_2$ limiting and resulting in a decreased photosynthesis rate (Levitt, 1972).

**Nutrient imbalance**

High levels of sodium (Na$^+$) and and other ions in salt-affected soils can induce nutrient imbalances of calcium, potassium, nitrate, magnesium, manganese, and phosphorus (Ca$^{2+}$, K$^+$, NO$_3^-$, Mg$^{2+}$, Mn, and P), causing deficiencies (Carrow and Duncan, 1998). High concentration of Cl$^-$ reduces NO$_3^-$ uptake by plants and high concentration of NO$_3^-$ inhibits phosphate uptake (Kozlowski, 1997). In soil with high exchangeable Na$^+$ content without adequate Ca$^{2+}$ and Mg$^{2+}$, soil permeability, which refers to the ability of water, oxygen, and roots to move within the soil macropores for good growth, decreases and several adverse soil physical conditions are exhibited (Carrow and Duncan, 1998). Potassium homeostasis is disrupted, possibly due to the ability of Na$^+$ to compete for K$^+$ binding sites. High ratio of Na$^+$ to K$^+$ and high concentrations of total ions inactivate enzymes and inhibit protein (Bartels and Sunkar, 2005).
**Ion toxicity**

Ion toxicity is the distinctive effects that occur when plants are subjected to salts for long periods of time. Generally, NaCl is the most common salt found in the ocean and soils. Low quality irrigation water containing high soluble salts and particular ions may be directly toxic to plants (Marcum, 2006). Toxicity may be expressed as direct toxicity to root tissues, and may accumulate to toxic levels in root and shoot tissues by continual root uptake (Carrow and Duncan, 1998). Plants vary considerably in leaf Na\(^+\) concentration that can cause apparent injury, depending greatly on species and cultivars within a species (Flowers and Yeo, 1986; Munns and Rawson, 1999). Many woody plants are relatively sensitive to Na\(^+\) toxicity. Accumulation of Na\(^+\) at leaf margins and tips causes dehydration and death. Accumulation of Cl\(^-\) in leaf tissues can lead to leaf burn and desiccation. Woody species are often more susceptible to Cl\(^-\) toxicity than most non-woody plants, while turfgrasses can tolerate higher Cl\(^-\) levels in soil (Carrow and Duncan, 1998).

**PLANT RESPONSES AND ADAPTATION TO SALINITY AND DROUGHT STRESSES**

**Mechanisms of adaptations**

Plant water deficits nearly always accompany droughts, but also occur at other times either because of excessive transpiration or when absorption is hindered by cold soil, soil salinity, or damage to root system (Pallardy, 2008). Plant adaptations to stresses are related to maintenance of plant water status (either water content: \(\psi_w\) or relative water...
content: RWC) during drought as illustrated in Fig. 1-1. An individual plant can exhibit several adaptations simultaneously or at different times during drought (Pallardy, 2008).

Drought avoiding plants occur in regions with well-defined dry seasons. They complete life cycles in a few weeks after rains and mature early in the summer before the soil dries (Pallardy, 2008). In other literature reviews, this mechanism could be called an escape mechanism (Fry and Huang, 2004).

Drought tolerant plants are described by the capacity of plants to pass through the active portion of their life cycle during periods when drought is expected (Fig. 1-1). Dehydration avoiding species have a large storage capacity and efficient control of the transpiration rate through reduced leaf size and altered morphology, leaf shedding, sunken stomata, abundant leaf waxes, a strong development of palisade mesophyll (Pallardy, 2008), and developing an extensive, deep root system to extract more water from a deeper and greater volume of soil (Huang and Gao, 2001; McCann and Huang, 2007). Dehydration tolerance is the capacity of protoplasm to sustain partial function or at least avoid irreversible injury as tissue $\psi_W$ declines. Tropical C$_4$ grasses and some woody plants from arid regions have appreciable protoplasmic tolerance of dehydration (Pallardy, 2008).

Desiccation avoidant plants maintain RWC even while $\psi_W$ falls, through at least 2 mechanisms, osmotic adjustment (OA) and elastic adjustment (EA). Desiccation tolerance mechanisms occur when RWC eventually falls to a critical level at which plant survival depends on the degree of dehydration that the protoplasm can endure without undergoing irreversible injury. At the extreme of cellular water loss are plants in which
vegetative parts can remain viable even when in equilibrium with water vapor in the air which present in lower plants such as bryophytes and mosses (Pallardy, 2008).

Fig. 1-1 General scheme of mechanisms of adaptation to drought. *Source*: Pallardy, 2008.

**Osmotic adjustment (OA)**

Osmotic adjustment (OA) is defined in the salinity paper as the osmotic pressure of plants adjusting to changes in the osmotic potential of the soil due to the salt concentration in the soil (Bernstein, 1961). Later the term OA is also used in the drought study to describe a decrease in plant osmotic potential through an increase in solute content or a decrease in water content in response to a decrease in external water potential to the extent that turgor potential is maintained (Shannon, 1997). OA is considered as an adaptation for surviving rather than for growing during stress periods (Munns, 1988). OA has been cited as a tolerance mechanism in both salt and drought
stress (Gupta and Berkowitz, 1987; Hinckley et al., 1980; Lemcoff et al., 2002; Munns, 1988; Suarez et al., 1998).

The degree of osmotic adjustment to drought and osmotically active compounds varies among species and genotypes. Sucrose, glucose, fructose, xylose, raffinose, and stachyose are accumulated in sugar maple leaves (*Acer saccharum*) (Wong et al., 2003). In grass species, glycinebetaine was positively correlated while proline concentration was negatively correlated with salinity tolerance in subfamily Chloridoideae (Marcum, 1999). In the *Eucalyptus* spp., cyclitols and carbohydrates are accumulated to osmotically significant concentrations in leaves (Merchant et al., 2006).

**Elastic adjustment (EA)**

Turgor can be influenced by changes in tissue elasticity. An elastic cell will sustain a smaller decrease in turgor potential as a given volume of water is lost than will a more rigid cell (Joly and Zaerr, 1987). Rigid cells allow a large difference in water potential between soil and leaves to be produced with relatively little water loss which would, in turn, increase water uptake (Bolaños and Longstreth, 1984). However, both high and low tissue elasticity are advantageous and contribute to turgor maintenance during drought stress depending on which response is favored in a particular condition (Fan et al., 1994; Joly and Zaerr, 1987; Pallardy, 2008; Zimmermann, 1978). A rigid cell wall will decrease in turgor pressure per unit of water loss more than an elastic cell wall; thus, its water potential is lower. As a consequence, soil-leaf water potential gradients increase and thereby promote water uptake from drying soil in order to maintain turgor pressure (Bowman and Roberts, 1985). In contrast, an elastic cell wall provides the cells
with a high resistance to short-term fluctuations and will sustain a smaller decrease in
turgor pressure as a given volume of water loss which contributes to turgor maintenance
(Joly and Zaerr, 1987; Zimmermann, 1978). However, changes in tissue elasticity may be
especially important for plants that do not show appreciable OA (Pallardy, 2008).

**Stomatal control via chemical and hydraulic signal**

It has long been apparent that the major plant growth regulating hormone abscisic
acid (ABA) strongly promotes stomatal closure (Schroeder et al., 2001) and can often
inhibit shoot growth (Wilkinson and Davies, 2002). Salinity and drought stresses induce a
significant ABA accumulation in roots (Jia et al., 2002; Lambers et al., 1998; Wang et al.,
2004) causing reductions in stomatal conductance, transpiration rate, and net
photosynthesis rate (Wang et al., 2004).

Isohydric and anisohydric behaviors have been used to describe the control of
stomata via hydraulic signal (Tardieu and Simonneau, 1998). In typical anisohydric
behavior, both leaf water potential ($\psi_{\text{leaf}}$) and stomatal conductance ($g_s$) decline with
decreasing soil water potential (drier soil). In contrast, isohydric species control gas
exchange in such a way that daytime leaf water potential does not depend on soil water
status (Lambers et al., 1998; Tardieu and Simonneau, 1998). In woody plants, upland
drought-tolerant species show anisohydric behavior adapted to fit in a water limited
environment by osmotic adjustment and by developing a deep and extensive root system,
sustaining the capacity for photosynthesis and resistance to protoplasmic injury. Riparian
species, generally considered drought sensitive, show isohydric behavior (Loewenstein
and Pallardy, 2002).
Salt exclusion and ion selectivity

Salinity tolerant species can avoid excess ions by excluding salt ions from the xylem of the roots (Schubert and Läuchli, 1990) or retaining ions in the root system and allowing the ions to be taken up the transpiration stream to the shoots for osmoregulation (Ball, 1988). Salinity tolerance is associated with shoot saline ion exclusion in grasses (Marcum and Pessarakli, 2006). Certain plants release excess salt through salt glands from which salts are either eliminated into the vacuoles of glands or secreted to the outside of the secretory cells (Kozlowski, 1997). Salt glands have been reported to occur in over 30 species in the Poaceae family (Marcum, 2007), as well as mangrove species (Kozlowski, 1997).

Salt compartmentalization

Species that cannot exclude most of the salt from the transpiration stream must also be able to compartmentalize the salt in vacuoles, thereby protecting the cytoplasm from ion toxicity and avoiding buildup in the cell wall which would cause dehydration (Munns, 2005). Mimura et al. (2003) found that vacuolar volume of mangrove (Bruguier sexangula) increased during the initial phases of salinity stress. Ion compartmentalization in the vacuole requires energy-dependent transport which is the cost to the plant of coping with stress (Hasegawa et al., 2000).
PLANT SPECIES AND THEIR HABITATS

Turfgrasses and woody species are selected based on habitats and photosynthetic pathway, which in all selected species is the C₃ pathway. Cool-season turfgrasses are commonly found in landscapes. Bigtooth maple and bigleaf maple are closely related to sugar maple but differ in habitats. Bigleaf maple is native to mesic-non saline habitats while bigtooth maple is native to more xeric-non saline habitats. Red gum is in mesic-saline habitats but expresses itself as a drought and salinity tolerant species. Thus, red gum is used as control check for the experiments.

Turfgrasses species

‘Matador’ Tall Fescue (PI 597935): *Schedonorus phoenix* (Scop.) Holub:

‘Matador’ TF was released in August 1996 by Pure Seed Testing, Inc. of Hubbard, Oregon. ‘Matador’ TF is a low-growing, high-density, dark green tall fescue that has an excellent establishment rate from seed and shows a good turf quality in temperate regions and has exhibited tolerance to stem rust and gray leaf spot (Fraser et al., 1999). ‘Matador’ TF was classified as a salt tolerant species using dosage required to kill 50% of the plants (Robins et al., 2009).

‘Midnight’ Kentucky bluegrass: *Poa pratensis* L:

‘Midnight’ KBG was developed by Pure Seed Testing, Inc. of Hubbard, Oregon using germplasm obtained from the New Jersey Agricultural Experiment Station.

‘Midnight’ KBG originated as a single, highly apomistic, aberrant plant. It is a persistent,
low growing, turf-type cultivar with the ability to produce a compact, dense turf with medium fine texture, a slow leaf extension rate, and a very dark green color. It has very good heat and cold tolerance, fair shade adaptation, a slow spring green up rate, and moderate fall low temperature color retention. It also possesses good establishment vigor, good mowing qualities, good tolerance of close mowing and a moderate nitrogen fertility requirement (Meyer et al., 1984). ‘Midnight’ KBG was classified as a moderately salinity tolerant species using dosage required to kill 50% of the plants (Robins et al., 2009).

Accession ‘S-107’ Kentucky bluegrass (PI372742): Poa pratensis L:

PI372742 accession was collected in Alaska, USA in 1972. It was classified as a salt tolerant species using dosage required to kill 50% of the plants (Robins et al., 2009). It is very fine textured with a light green color.

Accession ‘67-126’ Kentucky bluegrass (PI368233): Poa pratensis L:

PI368233 accession was collected in Alaska, USA in 1972. It was classified as a salt susceptible species using dosage required to kill 50% of the plants (Robins et al., 2009). It is very fine textured with a light green color.

**Woody plant species**

Bigtooth maple: Acer grandidentatum Nutt.

Small trees, mainly 4–8 m tall; herbage more or less villous to puberulent, at least on lower leaf surfaces. It is found with oak, oak-maple, sagebrush, Douglas fir, and white fir communities at 1280–2810 m in Utah, Idaho, Wyoming, Arizona, Mexico, and
Oklahoma. This plant is a principal component of the mountain brush community in Utah (Welsh et al., 1987). Total annual precipitation varies substantially from 258–454 mm (Bsoul et al., 2006; Phillips and Ehleringer, 1995). Though bigtooth maple grows best on deep soils, it can do well on shallower soils and drier sites, and is considered to be a good candidate for low water urban landscapes (Barker, 1977). It tolerates winter temperatures as low as -34°C. (Barker, 1977; Kuhns, 2010; Welsh et al., 1987), and is reported to be drought and salt tolerant (Emad, 2005).

Bigleaf maple: *Acer macrophyllum* Pursh.

Bigleaf maple is one of the few commercial hardwood tree species on the Pacific Coast. Most mature bigleaf maples are about 15 m tall and 50 cm in diameter at breast height. Large trees often reach heights of 30 m and diameters of 90–120 cm. The native range of bigleaf maple extends from latitude 33° to 51° N., always within 300 km of the Pacific Ocean. Bigleaf maple grows over a wide range of temperature and moisture conditions, from the cool, moist, marine climate of coastal British Columbia to the warm, dry, growing seasons of southern California. Springs, streams, and other permanent sources of water are often associated with bigleaf maple in southern California, but it also grows on eastern and northern slopes in California where more than 600 mm of annual rainfall occurs (Minore and Zasada, 2010).

This maple also grows on hot, dry sites in the central-western Cascade Range in Oregon and does not seem to be limited by moisture deficiencies there. Temperature probably limits the northern distribution of bigleaf maple. Douglas-fir, Pacific madrone, Pacific dogwood, swordfern, and prince’s-pine grow with bigleaf maple in most
environments. Bigleaf maple communities often present on moist sites include willow-back cottonwood-bigleaf maple and red alder-bigleaf maple/salmonberry. The bigleaf maple/snowberry community is found on dry sites. Bigleaf maple has a shallow, wide spreading system well suited to the shallow or saturated soils on which it often grows (Minore and Zasada, 2010). Bigleaf maple has been reported to be moderately drought and heat tolerant, but definitely shade tolerant (Sarr et al., 2011) and appears to be susceptible to salts (Dirkse, 2006).

Red gum: *Eucalyptus camaldulensis* Dehnh.

Red gum commonly grows to 20 m tall; occasionally reaching 50 m, with a trunk diameter of 1–2 m. Eucalyptus is phreatophytic and native to Australia and was described and named in 1788 by a French botanist. Red gum is one of the most widely distributed Eucalyptus species and is probably the world’s most widely planted tree in arid and semiarid lands. It is planted in many tropical and subtropical countries. Its natural distribution covers most of Australia’s mainland. Under natural conditions, red gum occurs typically along watercourses and on floodplains but occasionally extends to hills or ranges, from temperate to hot and from humid to arid zone. Red gum is often planted to assist the amelioration of saline areas (ICRAF, 2010). Its ability to tolerate salt and utilize saline ground water has been reported (Sun and Dickinson, 1995). This species is also drought tolerant due to its wide natural range across semiarid environments (Lemcoff et al., 2002). It has been reported to be both drought and salinity tolerant (Farrell et al., 1996; Gibson et al., 1994; Grieve et al., 1999; Merchant et al., 2006; Van der Moezel et al., 1988).
OVERALL HYPOTHESES AND OBJECTIVES

Hypotheses

1. Turfgrasses and woody species have somewhat common responses and mechanisms to cope with drought and salinity stress.

2. Drought tolerant plants are also salt tolerant and vice versa; thus, mesic-habitat plants, which are generally drought sensitive, show less salinity tolerance than xeric-habitat plants.

Objectives

1. Determine morphological and physiological responses of plants under salinity and drought stress conditions.

2. Understand the mechanisms that plants use to cope with salinity and drought stresses and compare those mechanisms among turfgrasses and woody species.

3. Determine the relationship between drought and salinity tolerant abilities in turfgrasses and woody species.

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CHAPTER 2

PHYSIOLOGICAL RESPONSES OF TURFGRASS SPECIES TO DROUGHT STRESS UNDER HIGH DESERT CONDITIONS

ABSTRACT

Broad concerns over water shortages and drought where irrigated urban landscapes are common in high desert regions have focused attention on drought tolerance of turfgrass species. We investigated the physiological responses of Kentucky bluegrass (KBG) and tall fescue (TF) under a prolonged drought under high desert conditions. The experimental design was a split plot with three replicates. Two irrigation treatments as a whole plot—well-watered and no-water—were applied to subplots of ‘Midnight’ KBG and ‘Gazelle’ TF. Stomatal conductance ($g_s$), canopy temperature, and predawn leaf water potential were measured over two seasons. KBG $g_s$ and leaf water potential decreased faster and to a greater extent than TF in response to soil drying, and KBG was in complete dormancy and brown within 5 weeks after cessation of irrigation. By contrast, TF maintained a green canopy throughout the drought periods. In the no-water plots, TF appeared to consume water from the deepest measured soil profiles (80 to 100 cm depth) while KBG used most of the water in the 50 to 60 cm depths. In late summer when watered for recovery, KBG plots were mostly green within 3 weeks after re-watering. The surface temperature of the well-watered plots was 6–13 °C cooler than the no-water plots and TF showed 5–7 °C lower temperature than KBG in no-water plots. TF is suitable for deep soil, exploiting a larger volume of water to avoid drought while

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KBG’s rapid drought avoidance would likely perform better in shallow landscape soils under drought.

**INTRODUCTION**

The Intermountain West (IMW) of North America is considered a high desert environment and is experiencing substantial population growth that means increasing demand for water, particularly due to irrigated urban landscapes (Kjelgren et al., 2000). Hot, dry summers are characteristic of the IMW, where urban turfgrass requires irrigation to survive, and thereby driving demand for water. However, the IMW has very limited water supplies, thus water conservation in irrigated urban landscapes has become an important policy to moderate consumption (Hilaire et al., 2008). Hydrological drought due to low winter snowpack is very common in the IMW and often leads to water conservation measures that can result in water stress of landscape plants, particularly turfgrass. Additionally, high temperatures and low humidity can increase drought stress on plants when irrigation is insufficient.

Drought tolerance refers to the ability to experience and undergo drought stress but survive (Fry and Huang, 2004). Plants adapted to water-limiting environments such as the IMW utilize a variety of adaptive mechanisms (McCann and Huang, 2007). Stomata control the exchange of water vapor and CO₂ between the interior of the leaf and the atmosphere, which contributes to control of the plant’s internal water status and to gaining carbon for photosynthesis (Hetherington and Woodward, 2003). Plants prevent water loss by closing stomata to reduce transpiration, but at the cost of reducing
evaporative cooling, increasing leaf temperature, and also decreasing photosynthesis and growth (Fry and Huang, 2004).

Stomatal closure with increased vapor pressure deficit of ambient air (VPD) is common in many plants to moderate transpiration under high evaporative demand (Bates and Hall, 1981; Monteith, 1995; Turner et al., 1984). More specifically, stomatal sensitivity is driven by leaf-to-air vapor pressure difference (LAVPD) for species in dense canopies (like turf) or with large leaves with boundary layers that limit convective heat loss (Landsberg and Butler, 1980; Montague et al., 2000; Turner, 1991). For such species, drought can trigger a feed-forward loop of progressively increasing LAVPD and stomatal closure until transpirational evaporative cooling is balanced by convective cooling (Jones, 1999). Progressive drought stress hastens this loop and increases stomatal sensitivity to LAVPD and correlates with decreased evapotranspiration rates (Al-Faraj et al., 2001).

Turfgrass species used in the IMW avoid drought but differ in mechanisms. Tall fescue (TF) avoids drought, as it maintains normal physiological function in water-limiting conditions by developing an extensive, deep root system to extract more water from a deeper and greater volume of soil (Huang and Gao, 2001). This postpones tissue dehydration (Sheffer et al., 1987). Tall fescue appears to also reduce water loss from transpiring leaves by rolling its leaves as soil water content declines (Qian and Fry, 1997). By contrast, Kentucky bluegrass (KBG) avoids drought by entering summer dormancy (Ervin and Koski, 1998), sometimes referred to as quiescence. But once adequate moisture is again available, plants will resume active growth (Laude, 1953).
Osmotic adjustment is another drought tolerance mechanism that grasses use to maintain cellular turgor and allow them to take up water at lower soil water potentials (Perdomo et al., 1996; White et al., 1992). Osmotic adjustment under stress conditions has been reported to occur in both TF (Qian and Fry, 1997; West et al., 1990; White et al., 1992) and KBG (Jiang and Huang, 2001; Perdomo et al., 1996).

Grass responses during prolonged summer drought have long been studied. Most researches have been conducted in greenhouses under controlled conditions (Aronson et al., 1987; Brown et al., 2004; Qian and Fry, 1997) while some were field investigations (Carrow, 1996; Laude, 1953; Richardson et al., 2008). Traits used to measure drought response have more commonly included morphological responses such as growth reduction, turfgrass quality rating, and root density (Ervin and Koski, 1998; Qian and Fry, 1997; Sheffer et al., 1987). Less often but more recently, physiological responses such as water relations, gs, photosynthesis, and hormone (ABA) concentration have been measured (Jiang and Huang, 2000; Perdomo et al., 1996; Volaire et al., 2009; West et al., 1990).

Since the mechanisms that KBG and TF use to cope with drought are quite different, a comparison under common field conditions, with detailed measurements, will help us understand the distinct drought tolerance or avoidance mechanisms utilized by these grasses. The objective of this work was to compare the physiological responses of KBG and TF, which differ in drought- coping mechanisms that might contribute to persistence of field-grown grasses during a prolonged drought in the IMW.
MATERIALS AND METHODS

Field plot

‘Midnight’ Kentucky bluegrass (KBG) (*Poa pratensis* L.) and ‘Gazelle’ tall fescue (*Schedonorus phoenix* (Scop.) Holub) were planted at the Greenville Research farm in North Logan, Utah (41° 45’ N and 111° 48’ W) in 2003. Temperature and precipitation data at the experimental site is summarized in Fig. 2-1. The soil at the experimental site was a silt loam, Millville series of uniform depth with a pH of 7.8-8.2 (Abdu et al., 2007). The experimental design was a split plot, with six main plots of 3 x 6 m each, divided into six subplots, each 1.5 x 2 m. Subplots were randomly assigned within each main plot and planted with a different turfgrass species.

Two irrigation treatments were applied to the whole plot area: (1) well-watered, irrigated three times a week with 1.2 cm of water, and (2) no-water plots that did not receive irrigation after June 12 in 2007 and June 25 in 2008. Well-watered plots were irrigated by hand to ensure uniformity and grasses were mowed at 7.62 cm and fertilized with ammonium sulfate at a rate of 9.8 kg ha⁻¹, which was applied per year to the plots—half in the spring and half in the fall.

Plots were four years old and well established when measurements were begun. In 2007, measurements were made twice each week from June 13 to August 22 then irrigation was resumed for 3 weeks to recover from prolonged drought. In 2008, measurements were conducted once a week from June 26 to August 7 then irrigated to allow recovery for 4 weeks.
Measurements

Predawn leaf water potential measurements were made twice a week in 2007 and once a week in 2008 using a pressure chamber (Model 3005HG, Soil, Moisture Equipment Corp, Santa Barbara, CA, USA). At predawn, five stems of each species in each subplot were collected by pulling the entire plant including the root, then immediately wrapping them in plastic wrap and storing them in a bag filled with ice for transport to the lab. Stems were cut slightly above the root and placed in the pressure chamber. Nitrogen gas was slowly applied to increase the chamber’s atmospheric pressure until water appeared at the cut end of the stem. The pressure reading was then taken and used as leaf water potential.

Stomatal conductance measurements were made twice a week in 2007 and once a week in 2008 using a leaf porometer (Model SC-1, Decagon Devices, Inc., Pullman, WA, USA). Measurements were taken between 11:00 AM and 2:00 PM on a clear day. Four to five blades of grass in each subplot were excised and arranged before clamping side by side with the adaxial side of the leaves facing the porometer chamber. Time used to prepare leaves for measuring after excising the leaves was less than 5 s to prevent the effect of water discontinuity on stomata. Stomata closure in Lucerne occurred within 2 h after cutting for hay and when the relative water content in tall fescue leaves remained between 80 – 90%, thus the stomatal conductance would not be affected by cutting (Harris and Tullberg, 1980). By using the instrument’s automatic mode, the $g_s$ was measured in 30 s. Eight separate measurements were made from each subplot and averaged for a final value.
Surface temperature of each subplot was measured using a digital thermometer (Model 52-II Dual Input Digital Thermometer, Fluke Corporation, Everett, WA, USA) connected with infrared temperature sensors (Model SI-111, Apogee Instruments, Inc., Logan, UT, USA) after measuring $g_s$ at 2:00 PM MDT. The infrared temperature sensor was held 1 m above canopy perpendicular to the ground allowing a field of view of 2 m in diameter.

Ambient air temperature data were continuously collected by a weather station in Greenville Research farm with a combination temperature and humidity sensor (model CR500, Campbell Scientific, Logan, Utah, USA). The sensor was scanned every 10 s and averages were recorded every 30 min with a datalogger (model CR1000, Campbell Scientific, Logan, Utah, USA). Vapor pressure deficit (VPD) and leaf-to-air vapor pressure difference (LAVPD) were calculated using ambient air temperature, dewpoint temperature, and leaf temperature as described by Murray (1967).

VPD is the difference between saturation vapor pressure and actual vapor pressure of ambient air ($e_s - e$) whereas LAVPD was calculated from the difference between saturation vapor pressure of the leaf using leaf temperature and actual vapor pressure of the ambient air ($e_l - e$).

Soil volumetric water content (VWC) was measured in 2008 using a frequency domain reflectometry (FDR) sensor (Diviner 2000, Sentek Sensor Technologies, Adelaide, Australia). One m long PVC tubes were installed in the center of each subplot. Every day at 4:00 PM, the Diviner 2000 probe was inserted into each access tube to measure water content to a depth of 100 cm (the deepest measurement of this study) with measurements made at 10 cm intervals.
Percent water use in each depth (10 cm interval) was calculated by the following equation:

\[
\% \text{ water use each depth (10 cm)} = \frac{VWC_{\text{initial}} - VWC_{\text{end}}}{\sum VWC_{\text{initial}} - VWC_{\text{end}}} \times 100%
\]

where \(VWC_{\text{initial}}\) is the volumetric soil water content at the beginning of the experiment; \(VWC_{\text{end}}\) is the volumetric soil water content at the end of the experiment.

**Statistical Analysis**

The experiment was a split plots design with six whole plots with two treatments applied (well-watered and no-water treatments) and three replicates of each. Treatment effects, species differences, and treatments x species interactions were determined by analysis of variance according to the Mixed procedure of SAS (version 9.0; SAS Institute, Cary, NC, USA). Thus, fixed parameters were species x treatments while random parameters were replicates, replicates x treatments, and replicates x species x treatment. Mean differences were tested with least significant difference test at a probability level of 0.05. Slope comparison was tested using GLM procedure of SAS.

**RESULTS**

The drought responses of the grasses varied slightly due to the difference in weather conditions between 2007 and 2008 (Fig. 2-1). Average air temperature in 2007 was about 1 to 2 °C higher than in 2008; however, average air temperature of both years was 2 to 3°C higher than the 30 year average. Moreover, total rainfall during both years was significantly lower than the 30 year average (Fig. 2-1). It should be noted that during
the study period, plants received some precipitation but it did not bring grasses out of dormancy. Recently, there was a study in the same field which indicated that it took greater than 13 mm of precipitation to restore active growth in turfgrass (unpublished data).

Fig. 2-1. Daily maximum and minimum air temperature, and precipitation in (a) a 30 year period (b) 2007 and (c) 2008.
As irrigation was withheld in the no-water plots, KBG entered summer dormancy in 5 weeks and all above ground tissues were brown, while TF remained green with some browning for the whole period of each experiment in both years. Each year, \( g_s \) of KBG in well-watered and no-water plots was equal at the beginning of the experiment but in the no-water plots, conductance decreased rapidly after irrigation stopped (Fig. 2-2).

Stomatal conductance differed from well-watered plots by week 1 in both years. Unlike KBG, \( g_s \) of TF in no-water plots was significantly lower than in well-watered plots by week 2 in both years. Irrigation was resumed on the no-water plots in August to end dormancy and restore active growth. The spike in Fig. 2-2e at day 48 was caused by about 8 mm of rainfall (Fig. 2-1b) stimulating a rapid increase in \( g_s \) in no-water TF but not in no-water KBG.

Following resumption of irrigation, \( g_s \) of no-water KBG equalized with that of well-watered plots after 3 weeks in 2007 and 4 weeks in 2008. TF recovered to the same degree of \( g_s \) after 2 weeks in 2007 and after 4 weeks in 2008. Overall, \( g_s \) of KBG dropped lower and faster than that of TF in both years. However, it took about the same length of time for KBG and TF to recover from prolonged drought.

Predawn leaf water potential (\( \psi_{\text{leaf}} \)) followed a similar trend to \( g_s \). As soil became drier, \( \psi_{\text{leaf}} \) of KBG was more negative and significantly lower than well-watered \( \psi_{\text{leaf}} \) by week 2 in both years. KBG entered complete summer dormancy when \( \psi_{\text{leaf}} \) dropped to -2.0 MPa. In contrast, the \( \psi_{\text{leaf}} \) of TF in no-water plots was significantly different from those in well-watered plots by week 3 in 2007 and by the first week in 2008. The sharp decrease of \( \psi_{\text{leaf}} \) in day 43 in 2008 (Figs. 2-2d and 2-2h) was due to the difficulty of measurements the week before (day 36) resulting in no data on that day, the initial week
Fig. 2-2. Stomatal conductance and predawn leaf water potential in well-watered and no-water plots of ‘Midnight’ KBG in (a,c) 2007 and (b,d) 2008, and of ‘Gazelle’ TF in (e,g) 2007 and (f,h) 2008. Well-watered plots are represented by a short dash line and no-water plots a solid line. The vertical line indicates irrigation resumption on the no-water plots. Letter ‘ns’ represents non significantly different ($P < 0.05$) while ‘*’ represents significantly different ($P < 0.05$) between pair of well-watered and no-water values at each day.
for KBG entering dormancy in 2007 (Fig. 2-2b). After the measurements for one week, the sudden drop of $\psi_{\text{leaf}}$ occurred. During the recovery period, $\psi_{\text{leaf}}$ of both species increased (less negative) to well-watered plot levels after 2 weeks of recovery in 2007 and 3 weeks in 2008.

Surface temperature at midday of no-water versus the well-watered plots was about 6–13 °C higher in both species, typical of drought stressed turf and manifested in a higher leaf and air temperature ($T_{\text{leaf}} - T_{\text{air}}$) difference in no-water than in well-watered plots ($P = 0.0008$) (Fig. 2-3a). $T_{\text{leaf}} - T_{\text{air}}$ in well-watered plots declined similarly in both species as vapor pressure deficit of ambient air (VPD) increased due to evaporative cooling. This well established inverse baseline relationship between $T_{\text{leaf}} - T_{\text{air}}$ and VPD for dense, uniform crop surfaces (Idso, 1982) has been conceptually refined (Blonquist et al., 2009) and applied to cool season turfgrass (Martin et al., 2005). In the no-water plots, drought-induced stomatal closure (Fig. 2-2a–b) reduced evaporative cooling in both species. However, KBG $T_{\text{leaf}} - T_{\text{air}}$ was higher than that of TF across all VPD levels as KBG entered dormancy and lost stomatal function.

In the well-watered plots, $g_s$ decreased as the LAVPD increased with no difference in slope ($P = 0.204$) of both species (Fig. 2-3b). As drought stress became more severe, LAVPD was greater due to stomatal closure, leading to an increase in leaf temperature, in turn causing greater differences in LAVPD (Fig. 2-3c). However, in no-water plots, the reduction in $g_s$ as LAVPD increased, indicated by the slope in Fig. 2-3c, was also not significantly different ($P = 0.313$) in both species at LAVPD $<5$ kPa. At LAVPD above 5 kPa, TF stabilized $g_s$ at about 150 mmol·m$^{-2}$·s$^{-1}$ whereas $g_s$ in KBG
dropped to lower levels and continued to decrease over a progressively higher range of LAVPD levels than TF until complete $g_s$ closure.

The rapid and progressive stomatal closure in KBG in response to soil drying is delineated more sharply when related to water potential (Fig. 2-4). The ratio of well-watered to no-water of leaf water potential versus $g_s$ of both species showed a similar trend, decreasing as drought-induced $g_s$ declined. This figure is similar to that of Kjelgren et al. (2009) but with the well-watered versus no-water relationship inverted. Stomatal conductance of TF in no-water plants declined along with declining leaf water potential but no-water $g_s$ did not fall below 30% of well-watered plants (ratio of well-watered to no water was not more than 3) while maintaining $\psi_{\text{leaf}}$ above 50% of well-watered plants. This trend was initially apparent in KBG as well, up to a well-watered to no-watered $g_s$ ratio of 2 (50% of well-watered plants), but over the space of a week the ratio increased to 4, as no-water fell to 25% of well-watered $g_s$. The ratio ultimately progressed to 10% (ratio of well-watered to no water was about 10) of well-watered plants, while $\psi_{\text{leaf}}$ only fell to 50% to 40% of well-watered plants, in contrast to TF, which maintained an apparent steady state balance between $g_s$ and $\psi_{\text{leaf}}$. KBG went dormant after stomata completely closed and leaf water potential stopped decreasing (more negative) whereas TF maintained open stomata, allowing a somewhat green canopy during the dry down period.
**Fig. 2-3.** The difference between air and leaf temperature over the range of VPD in well-watered of both species (solid line), in no-water ‘Midnight’ KBG (dash-dot-dot line), and in no-water ‘Gazelle’ TF (short dash line) (a); and the relationship between leaf-to-air vapor pressure difference (LAVPD) and $g_s$ in (b) well-watered plots and (c) no-water plots with the equation for LAVPD < 5 kPa. The $g_s$ at LAVPD > 5 kPa was shown in two lines where the short dash line represented ‘Gazelle’ TF and the dash-dot-dot line represented ‘Midnight’ KBG.
Fig. 2-4. The ratio of well-watered: no-water leaf water potential to $g_s$. Kentucky bluegrass lines divided into the $g_s$ ratio lower than 2 (a short dash line) and the $g_s$ ratio higher than 4 (a dash-dot-dot line) of $g_s$.

The greatest water use of plants in no-water plots was indicated by the greatest depletion to the lowest percent soil volumetric water content over the course of the study period (Figs. 2-5 and 2-6). KBG used more water from the soil at depths between 0 to 90 cm while TF used water down to 100 cm (greatest depth measured in this study) (Fig. 2-6); the large depletion at 100 cm suggested that TF likely extracted water below the measured root zone. Both species extracted more water at the surface (0 to 30 cm) than at the deeper soil profile, but TF depleted more water at the deepest depths than KBG.
Fig. 2-5. Volumetric soil water content at each 20-cm depth of well-watered plots of (a) ‘Midnight’ KBG, (b) ‘Gazelle’ TF, and of no-water plots of (c) ‘Midnight’ KBG, and (d) ‘Gazelle’ TF. The vertical line indicates irrigation resumption on the no-water plots. In no-water plots, the short dash line (40 to 60 cm) depth was the most deplete in volumetric soil water content in KBG (b) while the dotted line (80 to 100 cm) depth in TF decreased the most from the beginning of the experiment (d).
Fig. 2-6. Percent water use by plants in each 10 cm depth as a percent of the total water use (1 m depth) of ‘Midnight’ KBG and ‘Gazelle’ TF in no-water plots was calculated using the difference of soil water content between the start and the end of the experiment days before re-watering, and timed by the depth (10 cm) in each depth over the total from all depths (100 cm). The same letters are not significantly different (P < 0.05) in water use.

In addition, the total amount of water used by TF was 9% higher than by KBG. In order to better evaluate which depths contributed the most to variation in \( g_s \), stepwise regression was used to relate soil depth as independent variables (Xs) and \( g_s \) as the dependent variable (Y). In well-watered plots, the shallow depths (0 to 20 cm) contributed most to \( g_s \) in TF \( (F = 0.033 \text{ and } R^2 = 0.411) \) and at 60 to 80 cm depths in
KBG ($F = 0.014$ and $R^2 = 0.509$). However, the relationship of depth and $g_s$ in well-watered plants may not be meaningful due to low $R^2$. However in the no-water plots, the analysis indicated that the deepest depths (80 to 100 cm) are most important for TF regarding variation in $g_s$ ($F < 0.0001$ and $R^2 = 0.993$) while the 40 to 60 cm depth for KBG ($F = 0.0003$ and $R^2 = 0.991$) (Table 2-1). The stepwise regression supported evidence in Figs. 2-5 and 2-6 indicating that TF extracted more water from the deeper soil, and became water stressed when that layer was depleted, while KBG $g_s$ was more sensitive to soil water depletion in the top soil layer.

**DISCUSSION**

Turfgrass has limited stomatal control over transpiration due to low height and a thick boundary layer where the leaf surface is completely decoupled from conditions in the air outside boundary layer (Javis and McNaughton, 1986). Thus, the evapotranspiration continued even when stomata closure (Harris and Tullberg, 1980), in which water is plausibly lost through the cuticle (Cowan, 1977), resulted in rapid water depletion in soil.

As the soil dried, both species initially approached drought stress at the same rate, indicated from $g_s$ reduction (Figs. 2-2 and 2-4). In addition, both species did not moderate internal water potential through stomatal closure, which means the rate of water potential decline was rapid even when stomatal closed immediately after withholding irrigation. Rapid decline in water potential was likely due to greater boundary layer control over total transpiration than stomatal aperture (Zhang et al., 2007).
Table 2-1  Correlation efficiency and $P$ value of $g_s$ with volumetric soil water content at 20 cm interval depths.

<table>
<thead>
<tr>
<th>Species x Treatment</th>
<th>Soil depth (m)</th>
<th>0–0.2</th>
<th>0.2–0.4</th>
<th>0.4–0.6</th>
<th>0.6–0.8</th>
<th>0.8–1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-watered</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Midnight’ KBG</td>
<td>0.367 $^z$</td>
<td>0.546</td>
<td>0.673</td>
<td><strong>0.713 $^x$</strong></td>
<td>0.570</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.260 $^y$</td>
<td>0.082</td>
<td>0.023</td>
<td><strong>0.014</strong></td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Well-watered</td>
<td><strong>-0.642</strong></td>
<td>-0.488</td>
<td>-0.331</td>
<td>-0.019</td>
<td>-0.036</td>
<td></td>
</tr>
<tr>
<td>‘Gazelle’ TF</td>
<td><strong>0.033</strong></td>
<td>0.128</td>
<td>0.320</td>
<td>0.956</td>
<td>0.360</td>
<td></td>
</tr>
<tr>
<td>No-water</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Midnight’ KBG</td>
<td>0.905</td>
<td>0.980</td>
<td><strong>0.996</strong></td>
<td>0.967</td>
<td>0.955</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.034</td>
<td>0.003</td>
<td><strong>0.0003</strong></td>
<td>0.007</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>No-water</td>
<td>0.821</td>
<td>0.885</td>
<td>0.942</td>
<td>0.982</td>
<td><strong>0.997</strong></td>
<td></td>
</tr>
<tr>
<td>‘Gazelle’ TF</td>
<td>0.023</td>
<td>0.008</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td><strong>&lt;0.0001</strong></td>
<td></td>
</tr>
</tbody>
</table>

$^z$ Correlation coefficient

$^y$ $P$ value

$^x$ Bold type indicates the depth with the greatest contribution to changes in $g_s$ using stepwise regression.

Both species showed different responses when the soil dried and conditions became severe. KBG stomata seemed to be very sensitive to soil drying, as they closed more rapidly and absolute leaf water potential fell more rapidly than TF. KBG rapidly went dormant with all above ground tissues turning brown as a result from increasing $T_{leaf} - T_{air}$ (Fig. 2-3c) when $g_s$ fell below 50% of well-watered levels in potentially a feed-forward process (Fig. 2-3b). A small reduction in $g_s$ from soil drying will reduce transpiration cooling, increasing $T_{leaf} - T_{air}$ and LAVPD, and diminishing the boundary layer through increased eddy turbulence convection. Increased heating and decreased boundary layer in turn would push conductance even lower and LAVPD higher in a feed-
forward loop resulting in more and faster browning of tissues (Figs. 2-2 and 2-3). KBG appeared to reduce $\psi_{\text{leaf}}$ more than TF, suggesting that it can extract soil water at lower contents in the top layers to the point of triggering the feed forward stomatal closure cascade. At that point, the plants became dormant—a drought tolerance mechanism to avoid greater physiological damage to meristematic tissue and roots from water stress (Fry and Huang, 2004). As irrigation was resumed, the rapid resumption of growth occurred indicating that the rapid KBG dormancy allowed the meristematic growing points to survive under severe stress until the first fall rains coupled with cooler temperatures under natural field conditions (Laude, 1953). The physiological recovery time in no-water plots in our study was approximately 3 to 4 weeks after irrigation was resumed. Deeper rooting and maintenance of green foliage showed that TF did not reach the point over two growing seasons, one being exceptionally hot, where meristematic growing points were injured. Thus when the water resumed, rapid resumption of normal growth occurred almost at the same time as KBG.

TF, in contrast, could be classified as a drought evader (Fry and Huang, 2004). It kept the above ground tissues green because the deep root system allowed the plants to extract more water from deeper soil (Figs. 2-5 and 2-6). Initially TF responded to soil drying similarly to KBG, but reached a steady state between reduced transpiration and higher temperature (convective heat dissipation). Drought stressed TF thus maintained open stomata at about 30% of well-watered levels, and presumably continued photosynthesis that appeared to be in a steady state balance with water potential at about half of well-watered levels (Fig. 2-4) through extracting from increasingly deeper soil depths (Figs. 2-5 and 2-6).
In several studies, TF has been shown to have significantly more root mass, as measured by root length, at all depths under drought conditions compared to KBG (Ervin and Koski, 1998; Sheffer et al., 1987) and had 3 to 12 times greater root length in the lower profile (60 to 80 cm) in the field conditions (Su et al., 2008). This larger root system and greater ability to obtain water enables TF to maintain consistent rates of transpiration resulting in cooler surface temperatures (Fig. 2-3c) during drought compared to KBG.

Under well-watered conditions, both species showed similar responses to the high desert environments during summer. When water became limited, TF shows the ability to extract water from deeper in the soil profile as water content in the top layer depleted (Table 2-1 and Figs. 2-5 and 2-6). This allowed TF to maintain green leaves longer than KBG. TF relies on the deep root system to tolerate drought and may become fatally stressed in typically shallow urban landscape soils because the ability to avoid drought with deep rooting is lost. KBG, in contrast, goes dormant rapidly and preserves the growing point which makes it more suitable for shallow soil. However, its rapid feed-forward descent into dormancy in response to emergence of localized soil drying from non uniform irrigation application (Kjelgren et al., 2000) may result in over irrigation to avoid the browning of above ground tissue.

Under high desert conditions of the Intermountain West, TF has the ability to extract water from deep in the soil profile thereby minimizing irrigation as well as labor and other inputs associated with irrigation. Where water conservation and maintenance of green cover is the most important, TF may be better adapted than KBG, as long as TF is able to root deeply. As a result, above ground tissues of TF would stay green as long as
there is water in the soil profile. However, TF is likely to perform less well if the soil is shallow where its root system is not be able to penetrate deeply and soil water depletion would occur more rapidly. Therefore, in this case, KBG would be better suited because it would go dormant rather than suffer damage as TF would be liable to do.

REFERENCES


CHAPTER 3
SALINITY TOLERANCE IN TURFGRASSES
FROM GERMINATION TO MATURITY

ABSTRACT

Plants respond differently to salinity stress in stages of development from germination to maturity. Those responses are dependent on physiological mechanisms that differ among species and cultivars. The objectives of this study were to determine the salinity tolerance of turfgrass entries in different stages of growth and to study the physiological responses at maturity. ‘Midnight’ (Moderate KBG), accessions PI368233 (Tolerant KBG) and PI372742 (Susceptible KBG) Kentucky bluegrass (KBG) (Poa pratensis L.) and ‘Matador’ tall fescue (TF) [Schedonorus phoenix (Scop.) Holub] seeds were germinated in 1, 6, 12, 18, and 30 dS m$^{-1}$ for 28 days. As salinity increased, TF showed the highest germination percentage and growth rate followed by Tolerant KBG and Moderate KBG, while Susceptible KBG was the lowest in both. Germinated seeds were transferred to emergence trays. All KBG entries failed to emerge at high salinity dosage while TF was able to emerge. At maturity, TF, Tolerant KBG, and Moderate KBG grouped as tolerant to salinity while Susceptible KBG was intolerant. Based on 50% reduction in greenness, shoot and root dry weights, stomatal conductance, photosynthesis, and water use efficiency, Susceptible KBG showed the lowest salinity tolerance compared to the other entries. TF, Tolerant KBG, and Moderate KBG were clearly more tolerant than Susceptible KBG due to the ability to lower their $\psi_{\text{leaf}}$ and

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1 Coauthored by Nisa Leksungnoen, Paul G. Johnson, and Roger K. Kjelgren
maintain higher K⁺:Na⁺ ratio from root to shoot.

**INTRODUCTION**

Salinity of agricultural soils and poor management of irrigation leading to saline soils are major problems world-wide and have shaped civilizations for thousands of years (Jacobsen and Adams, 1958). Due to rapidly increasing costs of acquiring fresh water, diminishing supplies, and saving the high quality irrigation water for crop agriculture and more sensitive uses, low saline water is becoming an alternative source for irrigation, especially in large urban landscapes and golf courses in Texas, Colorado, California, Florida, and Arizona (Miyamoto and Chacon, 2006; Qian and Mecham, 2005; USEPA, 2004). As a result, many turfgrasses have been screened for salinity tolerance (Jing et al., 2008; Marcum, 2001; Robins et al., 2009; Torello and Symington, 1984) in hopes of developing more tolerant germplasm. Many have also been analyzed for growth responses to salinity stress (Alshammary, 2004; Horst and Beadle, 1984; Koch et al., 2011; Qian et al., 2001; Suplick-Ploense et al., 2002), and studied for salinity tolerance mechanisms (Marcum, 1999; Marcum, 2006; Marcum and Pessarakli, 2006; Poss et al., 2010; Qian et al., 2004; Torello and Rice, 1986).

Salinity stress is essentially physiological drought caused by high salt concentrations in the soil that limit water uptake because of low osmotic potential (Carrow and Duncan, 1998). This decreases capacity for photosynthesis (Munns, 2002) due to a reduction in leaf area (Taiz and Zeiger, 2006). Plants use different physiological mechanisms to cope with salinity stress. Ion exclusion from shoots is an important
mechanism to minimize toxic effects of salts (Marcum, 2007). For example, salinity
tolerance is negatively correlated with leaf Na\(^+\) concentration and positively correlated
with leaf salt gland Na\(^+\) excretion rate in bermudagrass (Marcum and Pessarakli, 2006).
Excretion of excessive toxic ions through trichomes, vesicular and glandular hairs
through leaf surface and retention of Ca\(^{++}\) and K\(^+\) in the shoot in stoloniferous arid zone
grass (*Aeluropus lagopoides*) appears to be an adaptive character of the salinity with
harsh desert conditions (Naz et al., 2009).

Levels of salinity tolerance among turfgrasses vary among and within species
(Qian et al., 2001; Torello and Symington, 1984). Some species are highly salinity
tolerant, such as seashore paspalum (*Paspalum vaginatum*), weeping alkaligrass
(*Puccinellia distans*), and inland salt grass (*Distichlis spicata*) (Marcum, 2007). Among
those used frequently as a turfgrass in the cool-season growing areas, tall fescue (TF) is
considered moderately salinity tolerant and more tolerant than Kentucky bluegrass
(KBG) (Alshammary et al., 2004; Marcum, 2007). However, some KBG accessions have
exhibited salinity tolerance equal to TF (Robins et al., 2009). KBG varieties in different
varietal classifications also differ in salinity tolerance. For example, compact and
aggressive types were more tolerant than common types, and there are also differences
within type as well (Qian et al., 2001).

KBG and TF have been frequently studied for salinity tolerance, and have shown
variation for tolerance at various stages of growth, specifically seed germination
(Harivandi et al., 1982; Horst and Taylor, 1983; Johnson et al., 2007; Lunt et al., 1961;
McCarty and Dudeck, 1993), seedling stages (Mueller and Bowman, 1989), and maturity
(Marcum, 1999; Qian et al., 2001; Torello and Symington, 1984). Perennial ryegrass
(Lolium perenne L.) is relatively tolerant to salinity during germination, but becomes more sensitive at later growth stages (Dudeck and Peacock, 1985). Most turfgrasses, including KBG and TF, are more tolerant during germination and less tolerant in seedling stages (Harivandi et al., 1982).

A few studies have been conducted on salinity tolerance at germination and seedling stage or germination and mature stage (Horst and Beadle, 1984; Jing et al., 2009). However, a combined study of seed germination, emergence (seedling stage), and mature stages using the same entries in cool-season grasses under saline conditions has not been reported. This comprehensive knowledge will help in planting management in order to know which stage is more tolerant, leading to more success of growth. Such information could also inform which stage is the most effective for selection of salinity tolerance.

This paper investigates salinity tolerance in KBG and TF from seed germination to maturity in order to determine differences in salinity tolerance among stages of growth and to explore tolerance mechanisms. In addition, we examine physiological responses of salinity tolerant entries versus salt sensitive entries. The objectives of this study were 1) to determine the salinity tolerance in different stages of growth, 2) to study the morphological and physiological responses to salinity stress of turfgrasses in mature stage, and 3) to identify the mechanisms in turfgrasses to cope with salinity stress.
MATERIALS AND METHODS

The experiments described below were conducted twice, once in 2008 and once in 2009. Differences between the two runs of the experiment are highlighted where necessary.

Three Kentucky bluegrass (KBG) varieties or populations and one tall fescue (TF) variety were chosen for this study. The variety ‘Midnight’ KBG was identified as moderately salinity tolerant (Meyer et al., 1984; Robins et al., 2009) and the National Plant Germplasm KBG accessions ‘S-107’ (PI372742) and ‘67-126’ (PI368233) were identified as salinity tolerant and intolerant respectively (Robins et al., 2009). ‘Matador’ TF was used as a salinity tolerant check (Robins et al., 2009). Therefore, Susceptible KBG, Tolerant KBG, Moderate KBG, and TF will refer to ‘67-126’ (PI368233), ‘S-107’ (PI372742), ‘Midnight’ KBG, and ‘Matador’ TF, respectively.

Germination stage

Fifty seeds of each entry were sterilized using 95% alcohol for 1 min and 2.0% Ca(OCl)₂ for 30 min (Stephenson, 1942) and placed on the germination blotter papers (Seedburo Equipment Company, Des Plaines, IL) which were placed in germination boxes and saturated with five salinity treatments—1, 6, 12, 18, 30 dS m⁻¹ of electrical conductivity (EC) solution. Salinity solutions were prepared from a mixture of NaCl and CaCl₂ at a 1:1 ratio (by weight) dissolved in deionized water. Three germination boxes per entry-treatment combination were used as replications.
Germination boxes were kept in a growth chamber with 8 hours warm (25°C)-light conditions and 16 hours cool (15°C)-dark conditions. Light intensity was set at 300 μmol m⁻² s⁻¹ with cool-white fluorescent light. Seeds were considered germinated when both shoots and roots were visible with 5–10X magnification (Johnson et al., 2007). Germinated seeds were counted every 2 days and removed to evaluate seedling emergence.

After 28 days, percent germination was calculated as well as germination rate using the formula described by Maguire (1962); Germination rate = ∑ (number of germinated seeds / days of counting). To evaluate viability of ungerminated seeds in the saline treatments, those seeds were placed on germination paper soaked with deionized water and observed for germination at 2-day intervals for 10 days.

This experiment was a completely randomized two factorial design with three replications. Germination percentage and rate were tested by analysis of variance (ANOVA) with the GLM procedure of SAS (version 9.0, SAS Institute, Cary, NC). Differences among means were tested with Fishers Protected LSD at a probability level of 0.05. Data was also fitted to a quadratic equation using SigmaPlot (version 11.0, Systat Software, Inc., Chicago, IL). The slope was used to compare an EC that caused 50% reduction of germination (Germination₅₀) using sums of squares with the Snedecor’s F statistic at a probability level of 0.05.

**Emergence stage**

The germinated seeds were transferred to container filled with 70-grit silica sand. Seeds were sowed in the sand at a depth of 1-cm. This is deeper than normal practice but
required to prevent seeds from floating away when the containers were immersed in the salinity treatment solution. In 2008, clear plastic boxes with clear lids were used as the sand media containers. The lids were used to help maintain constant salinity levels. However, the closed boxes allowed inside temperatures to rise to 40°C—too high for seed emergence. In 2009, 5.7-cm square plastic flats without covers were used to ensure suitable soil temperatures.

The emergence containers with holes in the bottom were immersed every other day into the appropriate salinity solution until saturation was reached, indicated by no bubbles emerging from the sand media. The salinity solutions were identical to those used in the germination experiments. Percentage of emergence was measured by counting the emergence of shoots from the sand for 28 days.

This experiment was a split plot design with salinity treatments (five levels) as the whole plot and species entries as subplots (four levels) with three replications. Seedlings germinated in the germination experiment were kept together as replications in the emergence experiment. Emergence rate was calculated in the same manner as germination rate in the previous experiment.

**Mature stage**

For the mature stage, we conducted two experiments: June to December, 2008 and July to November, 2009 using methods similar to Robins et al. (2009). Seeds of the same grasses described above were germinated with deionized water in boxes as described for the germination stage experiment. After 2 weeks, seedlings were transferred to containers (3.8- by 21-cm with 1.5-cm depth) (Ray Leach Cone-tainers, Stuewe and
Sons, Corvallis, OR) filled with 70-grit silica sand. The bottom of each container was plugged with capillary matting to confine the sand and slow the flow of water into the cones when immersed in a salinity solution tub during salinity treatments. During establishment, plants were overhead irrigated with nutrient solution (Peter's Excel Multi-Purpose 21-5-20 water soluble fertilizer with 100 ppm N; Everris, Camarillo, CA, USA) for 2 months prior to the initiation of the salinity treatments. Greenhouse temperatures were maintained at 25°C from 8:00 am to 4:00 pm and 15°C from 4:00 pm to 8:00 am.

Salt solutions used in these treatments were different from germination and emergence stage evaluations. A ratio of NaCl and CaCl₂ was used in the proportions described by Peel et al. (2004) to maintain a sodium adsorption ratio (SAR) of 3.5 in order to avoid an imbalance of Na⁺ in the salt solution. NaCl and CaCl₂ H₂O were weighed and mixed with nutrient solution until reaching the desired salinity levels; 1 dS m⁻¹ (EC of nutrient solution as control treatment), 6, 12, 18, and 30 dS m⁻¹.

Ten plants of each entry were randomly assigned to each of five salinity treatments and put in a rack. Thus, each rack (salinity treatment) contained 40 plants (four entries). This arrangement created a split plot design with salinity treatments as the whole plot and species-cultivar entries as subplots with three replications (three racks per entry-treatment combination). Similar to the emergence methods, the half racks were immersed into the appropriate salinity treatment solution tub until all air bubbles were gone, indicating soil saturation. In 2008, salinity concentration started at 3 dS m⁻¹ and increased in 3 dS m⁻¹ every week until the desired EC were reached (10 weeks for 30 dS m⁻¹). In 2009, the increment of salinity concentration was 6 dS m⁻¹ weekly (5 weeks for
30 dS m$^{-1}$). Salinity levels were increased incrementally to avoid physiological shock of high salinity to the plants (Peel et al., 2004).

Measurements

All measurements were conducted on the grasses after growing in each intended salinity treatment level for 2 weeks.

Leaf damage measurement. Three plants from each entry-treatment combination were randomly selected for digital photographs and digital image analysis weekly. Leaf damage was calculated based on the amount of green leaf tissue remaining after exposure to the salt treatments as calculated by a green-pixel counting procedure on the digital image (Crop Physiology Laboratory, USU, Logan, UT). A Nikon Coolpix 5400 digital camera was mounted on a tripod and placed 1 m from a background screen. All images were made in Auto mode using auto focus with center weighed metering, shutter 1/60, aperture F3.4, color balance AUTO, flash off, and 10 s self-timer in order to prevent movement when button was pressed. Photograph resolution in each image was 1,171,200 pixels (1280 x 915).

A series of macros were then used in Adobe Photoshop CS3 to automate the removal of the background in each image. Then, the “Magic Wand” tool allowed for the selection of a single color, within a specified tolerance range, which separated green from brown tissues—brown tissues being caused by the saline concentrations. For the small areas that macro could not delete, an eraser of 200-250 pixels in size was manually used to clean up all the remaining background color. The final processing used a program designed for counting pixels, in this case green pixels. After specifying a range of hue
and saturation values, the program automatically counted the number of green pixels out of the total in the image (1,171,200 pixels). Greenness was the percentage of green pixels divided by the total number of pixels in the image.

Leaf water potential ($\psi_{\text{leaf}}$). Five stems of each entry-treatment combination were cut slightly above the roots and placed into a pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Nitrogen gas was slowly applied to increase the chamber’s atmospheric pressure until water appeared at the cut end of the stems. That pressure was used as $\psi_{\text{leaf}}$. Five separate measurements were made from each entry-treatment combination and averaged for a final value of one replication.

Stomatal conductivity ($g_s$). Stomatal conductance ($g_s$) was measured with a leaf porometer (Model SC-1 Decagon Devices, Pullman, WA). Four to five intact blades of each entry-treatment combination were arranged with the adaxial side (top) facing to the open chamber. Multiple leaves were used and arranged without overlapping to ensure full coverage of the chamber. By using the instrument’s automatic mode, the $g_s$ was measured in 30 s. Five separate measurements were made from each entry-treatment combination and averaged for a final value of one replication.

Photosynthesis and water use efficiency (WUE). In 2009, we measured photosynthesis using a portable photosynthesis system (Model LI-6400, LI-COR, Lincoln, NE). An intact blade of each entry-treatment combination was placed into the fluorescence chamber. The instrument was set with the flow rate at 500 $\mu$mol m$^{-2}$ s$^{-1}$, CO$_2$ at 400 $\mu$mol m$^{-2}$ s$^{-1}$, and light at 800-1000 $\mu$mol m$^{-2}$ s$^{-1}$ with 10% of blue light. The data were manually logged after photosynthesis, CO$_2$, H$_2$O, and fluorescence were stable. These measurements were conducted over three days with one block (replication).
finished each day. Then, WUE was calculated using the equation (Li-6400 user’s manual guide):

\[
WUE = \frac{\text{Photosynthesis (} \mu \text{mol m}^{-2} \text{s}^{-1})}{\text{Transpiration rate (} \text{mol m}^{-2} \text{s}^{-1})} \times 100
\]

Shoot and root dry weights. After all measurements were done, plants were harvested by separating shoots and roots, then each was weighed and oven-dried at 80°C for 48 hours. Oven-dried shoots and roots of each entry-treatment combination were ground separately for ion concentration analysis. The ground samples were digested with nitric acid and 30% hydrogen peroxide and then analyzed for all cations, such as Na\(^+\), Ca\(^{++}\), and K\(^+\), with an inductively-coupled plasma spectrophotometer (ICP) (Model Iris Intrepid II, Thermo Scientific, Waltham, MA). For chloride, ground samples were digested with 2% acetic acid and then analyzed with a flow injection analyzer (Model Lachat Quickchem 8000 series method 10-117-07-1-C, Lachat instruments, Loveland, CO).

Statistical analysis

The experiment was a split plot design with three blocks (replications) of five main plots (salinity level treatments) and four subplots (grass entries) and ten replications within each block that averaged to get one final value. Data were subjected to ANOVA and tested for salinity effects, grass entry effects, and interactions using the Proc Mixed of SAS (version 9.0, SAS Institute, Cary, NC). Fixed parameters were entries x treatments and random parameters were replications, replications x treatments, and replications x entry x treatment. Differences among means were tested with Fishers
Protected LSD at a probability level of 0.05. Data was also fitted to a quadratic equation using SigmaPlot (version 11.0, Systat Software, Inc., Chicago, IL) in order to compare the slope of an EC that caused 50% reduction in greenness (Greenness₅₀), shoot (Shoot₅₀), root (Root₅₀), stomatal conductance (gₛ₅₀), and photosynthesis (Photo₅₀) using sums of squares with the Snedecor’s $F$ statistic at a probability level of 0.05.

RESULTS

Salinity decreased the germination rate, emergence rate and also photosynthesis rate in all entries in this study. At maturity, leaf burn symptoms were commonly found in all entries at high salinity concentration, but it was more pronounced in Susceptible KBG than other entries. TF, Tolerant KBG, and Moderate KBG performed better than Susceptible KBG in both morphological and physiological assessments under salinity stress.

Germination stage

Salinity reduced both the percent germination and slowed germination rate. Seeds of all entries started germinating by day 6 in the control, 6, and 12 dS m⁻¹ treatments, but delayed to day 8 in the 18 dS m⁻¹ treatment in both years and by day 18 in 2008 and day 10 in 2009 in the 30 dS m⁻¹ treatment (Figs. 3-1 and 3-2). Salinity reduced the germination rate as shown by a decrease in the slope of percent germination. Of all entries, Susceptible KBG exhibited the slowest germination rate in all salinity levels in
Table 3-1  Electrical conductivity that caused 50% reduction in percent germination (Germination$_{50}$), percent emergence (Emergence$_{50}$), Greenness (Greenness$_{50}$), shoot dry weight (Shoot$_{50}$), root dry weight (Root$_{50}$), stomatal conductance (gs$_{50}$), and photosynthesis (Photo$_{50}$) in 2008 and 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>Germination$_{50}$</th>
<th>Emergence$_{50}$</th>
<th>Greenness$_{50}$</th>
<th>Shoot dry weight$_{50}$</th>
<th>Root dry weight$_{50}$</th>
<th>Stomatal conductance$_{50}$</th>
<th>Photosynthesis$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF</td>
<td>24.7</td>
<td>37.4a$^y$</td>
<td>-</td>
<td>11.5</td>
<td>7.9</td>
<td>24.3</td>
<td>19.8a</td>
</tr>
<tr>
<td>TOL</td>
<td>20.2</td>
<td>23.4b</td>
<td>-</td>
<td>6.3</td>
<td>7.2</td>
<td>20.3</td>
<td>9.7a</td>
</tr>
<tr>
<td>MOD</td>
<td>15.4</td>
<td>24.0b</td>
<td>-</td>
<td>5.8</td>
<td>7.4</td>
<td>20.8</td>
<td>11.3a</td>
</tr>
<tr>
<td>SUS</td>
<td>13.2</td>
<td>16.6c</td>
<td>-</td>
<td>4.0</td>
<td>5.7</td>
<td>12.9</td>
<td>3.8b</td>
</tr>
<tr>
<td>Significant$^x$</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>***</td>
</tr>
</tbody>
</table>

$^z$Turfgrasses species; TF = ‘Matador’ tall fescue, TOL = Tolerant Kentucky bluegrass, MOD = Moderate Kentucky bluegrass, and SUS = Susceptible Kentucky bluegrass

$^y$Electrical conductivity in each column followed by the same letter are not significantly different at the $P \leq 0.05$.

$^x$The symbols *, **, and *** are used to show significance at the $\alpha = 0.05$, 0.01, and 0.001 levels, respectively. NS is used to show no significance at the $\alpha = 0.05$. 

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Fig. 3-1. Percent seed germination under salinity stress over time in 2008 of ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG).
Fig. 3-2. Percent seed germination under salinity stress over time in 2009 of ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG).
both years followed by Moderate KBG and Tolerant KBG, while TF had the fastest germination rate (Figs. 3-1 and 3-2).

The solution EC that caused a 50% reduction in seed germination percentage (Germination_{50}) (Table 3-1 and Fig. 3-3) indicated that TF was the most salinity tolerant based on Germination_{50} while Susceptible KBG was the least tolerant. Moderate KBG and Tolerant KBG Germination_{50} were intermediate and not significantly different from each other.

![Graph showing seed germination percentage vs. solution electrical conductivity (dS m^{-1})](image)

**Fig. 3-3.** Seed germination of salinity plants relative to control plants of ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ’67-126’ (PI368233) (Susceptible KBG) in (a) 2008 and (b) 2009. The same letters are not significantly different slopes that fit with the quadratic equation at \( P = 0.05 \). NS means that there is no significant difference among entries in slopes of germination percentage.
When ungerminated seeds in the saline treatments were removed to non-saline conditions, germination reached near the levels of the control (data not shown) which means the seeds were viable but not able to germinate in the conditions.

**Emergence stage**

Like germination, emergence decreased as salinity increased. Unfortunately, data in 2008 were inadequate to make conclusions due to the effects of high soil temperature. Thus, only 2009 data were used for analysis. Percent emergence in TF was clearly distinguishable from KBG in Fig. 3-4. However, solution EC that caused a 50% reduction in emergence \((\text{Emergence}_{50})\) was not different among entries. None of the KBG entries could emerge at 18 dS m\(^{-1}\) even though seeds could germinate at this concentration. TF, in contrast, exhibited about 30% emergence at 18 dS m\(^{-1}\).

**Mature stage**

Leaf damage measurement

Salinity damage as observed on the leaves was obtained based on percent green pixels in the image of treated plants relative to control plants (Fig. 3-5). Leaf damage increased (less greenness) as salinity concentration increased. However, there was no difference among entries in terms of an EC that caused a 50% reduction in green pixels \((\text{Greenness}_{50})\) (Fig. 3-6a-b) which indicated visual quality under salinity treatments (Table 3-1). Greenness in 2008 was obviously lower than in 2009, with the discrepancy possibly due to the difference in length of time when salinity treatments were applied. In
2008, plants received salinity treatments for 10 weeks compared to 5 weeks in 2009 which created a more rapid increase to the target salinity levels. The 10-week duration in 2008 was perceived as too long and possibly confounding salinity concentration with exposure time resulting in low greenness.

Fig. 3-4. Emergence as percent of salinity plants relative to control plants of ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG) in 2009. NS means that there is no significant difference among entries in slopes of emergence percentage.
**Fig. 3-5.** Digital images of ‘Matador’ tall fescue (TF) in the (a) control treatment, (b) 30 dS m\(^{-1}\) treatment, (c) control treatment after removal of the background, and (d) 30 dS m\(^{-1}\) after removal of the background. Background removal in the images was done by Photoshop prior to processing the pictures to count the green pixels.

Shoot and root dry weights

Dry weights of the plants decreased as salinity increased (Fig. 3-6c–f). Overall, Moderate KBG showed the highest dry weights followed by TF, Tolerant KBG, and finally Susceptible KBG (Table 3-1). Interestingly, TF had a similar Shoot\(_{50}\) and Root\(_{50}\) in both years, while KBG entries were higher in 2009 than in 2008. Exposure to the salt stress did not seem to affect TF as much as KBG. Root:shoot ratio was not significantly different among treatments but was different among entries (Fig. 3-6g–h). TF exhibited the highest ratio while Moderate KBG showed the lowest \((P < 0.001)\) in both years. Tolerant KBG and Susceptible KBG were intermediate and not significantly different
from each other in 2009 ($P = 0.8821$).

Leaf water potential

Leaf water potential ($\psi_{\text{leaf}}$) decreased (more negative) as salinity increased (Fig. 3-7). Susceptible KBG had higher $\psi_{\text{leaf}}$ (less negative) than other entries at high salinity levels, especially in 2009; it did not adjust osmotic potential. Moderate KBG showed some adjustment of $\psi_{\text{leaf}}$ at lower concentrations (6 dS m$^{-1}$), but not at higher salinity levels. In comparison, Tolerant KBG and TF showed adjustment of $\psi_{\text{leaf}}$ in 12 dS m$^{-1}$, then equal levels to 30 dS m$^{-1}$.

Stomatal conductance, photosynthesis, and water use efficiency (WUE)

High salinity induced stomatal closure (Fig. 3-8a-b). However, an EC that caused a 50% reduction in $g_s$ ($g_{s50}$) was not different among entries in both years (Table 3-1). Photosynthesis in Susceptible KBG was lower than other entries even though $g_s$ was not different (Fig. 3-8c) suggesting that salinity concentration interfered with photosynthesis and not solely due to stomatal closure. TF showed the greatest WUE at high salinity levels, followed by Moderate KBG and Tolerant KBG (Fig. 3-8d). TF is producing more photosynthate, and therefore more biomass, than all three KBG entries at a given degree of stomatal aperture (water loss). In addition, TF and Tolerant KBG showed the highest WUE at mild stress (12 dS m$^{-1}$) than control treatment indicating the ability to mediate growth under stress condition.
Fig. 3-6. Growth measurements expressed as percent of control for ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG) at salinity levels of 6, 12, 18, and 30 dS m$^{-1}$ in (a) greenness in 2008 and (b) greenness in 2009, (c) shoot dry weight in 2008, (d) shoot dry weight in 2009, (e) root dry weight in 2008, (f) root dry weight in 2009, (g) root:shoot ratio in 2008, and (h) root:shoot ratio in 2009. Lines with the same letters have slopes modeled by a quadratic equation that are not significantly different at $P = 0.05$. 
Solution electrical conductivity (dS m$^{-1}$)

<table>
<thead>
<tr>
<th>Root:Shoot ratio</th>
<th>Tall fescue</th>
<th>Tolerant KBG</th>
<th>Moderate KBG</th>
<th>Susceptible KBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(a) Greenness 2008</td>
<td>(b) Greenness 2009</td>
<td>(c) Shoot dry weight 2008</td>
<td>(d) Shoot dry weight 2009</td>
</tr>
<tr>
<td>0.2</td>
<td>(e) Root dry weight 2008</td>
<td>(f) Root dry weight 2009</td>
<td>(g) Root:Shoot ratio 2008</td>
<td>(h) Root:Shoot ratio 2009</td>
</tr>
<tr>
<td>0.4</td>
<td>(a) Greenness 2008</td>
<td>(b) Greenness 2009</td>
<td>(c) Shoot dry weight 2008</td>
<td>(d) Shoot dry weight 2009</td>
</tr>
<tr>
<td>0.6</td>
<td>(e) Root dry weight 2008</td>
<td>(f) Root dry weight 2009</td>
<td>(g) Root:Shoot ratio 2008</td>
<td>(h) Root:Shoot ratio 2009</td>
</tr>
<tr>
<td>0.8</td>
<td>(a) Greenness 2008</td>
<td>(b) Greenness 2009</td>
<td>(c) Shoot dry weight 2008</td>
<td>(d) Shoot dry weight 2009</td>
</tr>
<tr>
<td>1.0</td>
<td>(e) Root dry weight 2008</td>
<td>(f) Root dry weight 2009</td>
<td>(g) Root:Shoot ratio 2008</td>
<td>(h) Root:Shoot ratio 2009</td>
</tr>
</tbody>
</table>

50% Reduction in greenness

50% Reduction in shoot dry weight

50% Reduction in root dry weight
Ratio of stomatal closure and leaf water potential

A greater slope of the relationship between ratio of stress over control in g_s and \( \psi_{\text{leaf}} \) in both years (Fig. 3-9) indicated a better chance of plants extracting more water from soil as stomata continued to close. Thus, TF exhibited the lowest \( \psi_{\text{leaf}} \) at the same g_s compared to all three KBG entries (\( P = 0.0008 \)), suggesting that TF could extract more water from soil than KBGs making it a better adjustment to salinity than KBG entries. Among KBG entries, Tolerant KBG showed the greatest slope, Susceptible KBG was lowest, while Moderate KBG was intermediate. The slope for Susceptible KBG was almost flat in 2009 (Fig. 3-9b), indicating an inability to extract water at higher salinity.

![Fig. 3-7. Leaf water potential expressed as percent of control for ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG) at salinity levels of 6, 12, 18, and 30 dS m\(^{-1}\) in (a) 2008 and (b) 2009.](image-url)
Fig. 3-8. Physiological measurements expressed as percent of control for ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG) at salinity levels of 6, 12, 18, and 30 dS m\(^{-1}\) in (a) stomatal conductance in 2008 and (b) stomatal conductance in 2009, (c) photosynthesis in 2009, and (d) water use efficiency in 2009. The same letters are not significantly different slopes that fit with a quadratic equation at \( P = 0.05 \) in photosynthesis. Lines with the same letters have slopes modeled by a quadratic equation that are not significantly different at \( P = 0.05 \).
Tissue ion concentration distribution

The distribution of sodium (Na\(^+\)) and potassium (K\(^+\)) from roots to shoots as a response to salinity levels was shown as shoot:root ratio of the ions (Fig. 3-10a–b). There were two distinguishing groups with regards to Na\(^+\) transport from the roots to shoots \((P = 0.010)\) (Fig. 3-10a). In TF and Tolerant KBG, Na\(^+\) levels in the shoots were not changed at higher salinity compared to the control treatments (100%). In contrast, Moderate KBG and Susceptible KBG showed higher levels of Na\(^+\) in the shoots in the higher salinity treatments.

In contrast to the generally rising levels of Na\(^+\) levels at higher salinity levels, K\(^+\) in the shoots decreased compared to the roots (Fig. 3-10b). Among all entries, Moderate KBG showed the highest K\(^+\) shoot:root ratio followed by TF and Tolerant KBG, which exhibited similar rates of K\(^+\) transportation. Susceptible KBG showed the lowest levels of K\(^+\) movement \((P = 0.0005)\) from roots to shoots.

K\(^+\):Na\(^+\) ratio followed the similar trend as K\(^+\) in which the ratio decreased as salinity increased (Fig. 3-10c). Susceptible KBG had significantly lower K\(^+\):Na\(^+\) shoot:root ratio than other entries \((P = 0.003)\). Other ions, including calcium (Ca\(^{++}\)) and chloride (Cl\(^-\)), increased in shoots as salinity increased (data not shown). However, there was no differences in tissue concentrations among entries \((\alpha = 0.05)\) in this study.
**DISCUSSION**

Our results suggest that all four entries tested here were more salinity tolerant at germination, less tolerant at emergence, and more tolerant when plants were mature (Table 3-1). Wang and Zhang (2011) observed similar salinity tolerance at germination and in mature plant stages in ‘Falcon IV’ TF and ‘Langara’ and ‘Park’ KBG as measured by Germination$_{50}$ and Shoot$_{50}$ compared to control. However, salt tolerance in grasses is
Fig. 3-10. Ratio of shoot to root in tissue concentration expressed as percent of control for ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG) at salinity levels of 6, 12, 18, and 30 dS m⁻¹ in (a) Na⁺, (b) K⁺, and (c) K⁺:Na⁺. Lines with the same letters have slopes modeled by a quadratic equation that are not significantly different at P = 0.05 of ‘Matador’ tall fescue (TF), ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and ‘67-126’ (PI368233) (Susceptible KBG).
inconsistent over developmental stages among species and cultivars, making generalizations difficult. For example, Bernstein and Hayward (1958) and Horst and Beadle (1984) report generally more sensitivity during germination. In contrast, Italian ryegrass (*Lolium multiflorum* L.) is relatively tolerant during germination but less tolerant during maturity (Marcar, 1987).

**Germination and emergence stage**

The results indicate that total germination and germination rate under salinity stress differed among species and entries (Fig. 3-3) plausibly from genetic differences among them (Camberato and Martin, 2004; Tarasoff et al., 2009; Wang and Zhang, 2010). Larger seed size of TF may also play an important role in the ability to germinate and emerge faster and at a greater percentage under high salt concentration than KBG (Jackson et al., 1992; Wu and Du, 2007). However, Newell and Bludau (1993) observed that there is no association between seed size and germination in KBG cultivars. Seeds that did not germinate in the saline treatments were still viable; therefore, high salinity suppressed their germination (Marcar, 1987). At high salinity, seeds absorbed little water due to the low water potential (Atia et al., 2011; Marcar, 1987). Emergence of newly germinated seedlings appeared more sensitive to salinity than germination. However, sowing depth could explain the low emergence percentages exhibited by the entries in this study.
Mature stage

Salinity tolerance in mature stage of grasses in our study was based on both morphological (Greenness$_{50}$, Shoot$_{50}$, Root$_{50}$, and root:shoot ratio) and physiological ($\psi_{\text{leaf}}$, $g_{50}$, Photo$_{50}$, WUE, and $K^+:Na^+$ ratio) criteria. Overall, there were two distinct groups of entries. The tolerant group included TF, Tolerant KBG, and Moderate KBG while Susceptible KBG was the intolerant group. Among the tolerant group, TF was the most tolerant. Tolerant KBG and Moderate KBG were less salinity tolerant than TF, but equal to each other in this experiment. Previously, Moderate KBG was reported to be less salinity tolerant than Tolerant KBG (Robins et al., 2009).

Salinity stress is a desiccation stress, and therefore salinity tolerant plants can tolerate (or avoid) this desiccation. In theory, plants under stress have a higher root:shoot ratio than those under optimal conditions because more photosynthate is directed to root production than to shoots (Kozlowski and Pallardy, 2002). However, experimental work has not always followed. For example, Alshammary et al. (2004) reported no change in root:shoot ratio in response to salinity. Likewise, our study indicated no difference in root:shoot ratio between control and salinity treatments. Increasing root mass over shoot mass in order to deal with salinity stress was not an apparent salt tolerance strategy of TF and the KBG entries in this study.

Plants open their stomata to create a $\psi_{\text{leaf}}$ gradient between the leaf and the atmosphere which enables movement of water from soil to leaves (Taiz and Zeiger, 2006). In order to absorb water from soil, including saline conditions, root water potential must be lower than the soil solution resulting in further lower water potential in leaves.
Salts cause lower water potential (more negative) in soil solution creating the desiccation. However, a tradeoff exists between acquiring CO₂ for photosynthesis and losing water through transpiration (Taiz and Zeiger, 2006). The tolerant entries—TF, Tolerant KBG and Moderate KBG—continued opening stomata along with adjusting their $\psi_{\text{leaf}}$ lower (Fig. 3-9), resulting in more photosynthesis (Fig. 3-8c) and WUE (Fig. 3-8d). Susceptible KBG, in contrast, appeared to keep stomata open to create $\psi_{\text{leaf}}$ gradient (Fig. 3-8a) but could not lower its $\psi_{\text{leaf}}$ (Fig. 3-7) causing less water transport from roots to leaves resulting in desiccation (Reina-Sánchez et al., 2005; Verslues et al., 2006) and lower greenness (Fig. 3-6a-b).

Mild salinity (12 dS m⁻¹) increased WUE in TF and Tolerant KBG (Fig. 3-8d). This could be because as salinity increased, the reduction in stomata opening (50%) (Fig. 3-8a-b) was less than for photosynthesis (80%) (Fig. 3-8c), indicating less water loss compared to CO₂ uptake resulting in high WUE. In addition, their $\psi_{\text{leaf}}$ were obviously low at 12 dS m⁻¹ (Fig. 3-7) indicating that more water was extracted from soil into plant tissues, possibly to be used in photosynthesis processes rather than spent in transpiration due to stomatal closure in this study. High WUE under mild salinity stress has also been observed in wheat (Shaheen and Hood-Nowotny, 2005), sugar beet and cowpea (McCree and Richardson, 1987), and perennial halophyte (Sesuvium portulacastrum) (Slama et al., 2008).

$K^+ : Na^+$ ratio is a well-known criteria for determining salinity tolerant species (Flowers and Yeo, 1986; Munns and Rawson, 1999; Peng et al., 2004), with a higher $K^+ : Na^+$ ratio indicating greater tolerance in many species (Krishnan and Brown, 2009;...
Qian et al., 2000; Qian et al., 2001). TF and Tolerant KBG showed the highest root to shoot K:Na ratio (Fig. 3-10c) due to a restriction of Na\(^+\) movement from roots to shoots in the most salinity tolerant entries (Fig. 3-10a). Moderate KBG allowed Na\(^+\) from roots to move past to shoots (Fig. 8a) but a great amount of K\(^+\) was also transported to shoots as well (Fig. 3-10b) resulting in moderate K\(^+\):Na\(^+\) ratio. Susceptible KBG allowed Na\(^+\) to accumulate in shoots but did not have the ability to transport K\(^+\) causing the low K\(^+\):Na\(^+\) ratio.

The difference between the salinity tolerant and intolerant group in our study was largely attributed to high root:shoot ratio (Fig. 3-6g–h), sodium exclusion at roots level (Fig. 3-10a), and high K\(^+\):Na\(^+\) ratio (Fig. 3-10c). This is similar to Qian et al. (2001) where a difference in growth was observed between two KBG cultivars (‘Limousine’ and ‘Kenblue’) under salinity. The difference in salt uptake in each entry probably resulted from the K\(^+\):Na\(^+\) selectivity of the plasma membrane (Peng et al., 2004). In a paper by Krishnan and Brown (2009), red fescue accessions FR1 and FR2 were shown to have the ability to exclude Na\(^+\) from within shoots from younger leaves to older leaves, while perennial ryegrass utilizes Na\(^+\) exclusion from the xylem stream at the root apoplast and maintains the higher K\(^+\):Na\(^+\) ratio.

Our results suggest that emergence was the most vulnerable stage of growth in these entries we tested. Seeds could germinate under moderately high salinity stress, but the emergence phase appeared more sensitive, which in a field application would reduce the stand of seedlings. Thus, salinity level defined as emergence\(_{50}\) would be important to consider when planting in salt-affected areas. Morphological criteria such as survival
rate, growth rate, leaf area, leaf injury, and root:shoot ratio will be the next important
criteria for the seedling stage. Physiological criteria will be more important for mature
plants because of the long-term exposure to salinity stress they experience. Plants need to
maintain physiological mechanisms such as $\psi_{\text{leaf}}$, transpiration rate, and photosynthesis
rate in order to continuously grow under stress and survive to provide a functional
turfgrass stand.

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CHAPTER 4

SALINITY TOLERANCE IN WOODY SPECIES DIFFERING IN NATIVE SALINITY AND WATER AVAILABILITY HABITATS

ABSTRACT

Salt dose responses of three tree species differing in water availability and salinity habitats were compared. *Acer grandidentatum* Nutt. (bigtooth maple; xeric-non saline), *A. macrophyllum* Pursh. (bigleaf maple; mesic-non saline) and *Eucalyptus camaldulensis* Dehnh. (red gum; mesic-saline) was compared to assess if salinity tolerance could be inferred from water availability habitat. Five levels of salinity solution (control, 3, 6, 9, 12 dS m\(^{-1}\)) were applied using an adapted low volume near-continuous gradient dosing system. Physiological responses measured weekly were stomatal conductance, leaf water potential, and photosynthesis after increasing salt application to target salinity levels, and pressure-volume curves taken from control and 12 dS m\(^{-1}\) treatments. Salinity impact on leaf appearance was measured photographically and in changes in green leaf area. Eucalyptus was most saline tolerant by an order of magnitude than the maple species, but bigtooth maple exhibited greater salt tolerance than bigleaf maple. Each species responded to salinity stress with different mechanisms. Eucalyptus exhibited root-level salt exclusion and leaf-level osmotic adjustment. Bigtooth maple maintained turgor pressure via increasing cell wall elasticity and apoplastic water fraction. Bigleaf maple failed to exclude salts from tissue, accumulating to toxic levels that causing severe leaf

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1 Coauthored by Nisa Leksungnoen, Roger K. Kjelgren, Paul G. Johnson, Grant E. Cardon, Richard C. Beeson, Jr., and Austin Hawks
damage, and had no ability to maintain turgor pressure. The somewhat greater salinity tolerance of bigtooth maple suggests that species from xeric habitats would likely have inherently greater salt tolerance than those from mesic habitats, but not to the level of species clearly adapted to saline conditions.

**INTRODUCTION**

Salt limitations on plant growth have been recognized since an ancient Mesopotamian times (Ghassemi et al., 1995; Jacobsen and Adams, 1958). All soils contain some soluble salts, but when soil and environmental conditions allow concentration in top soil layers that impacts plant production and health, then salinity becomes an issue of land degradation (Rengasamy, 2006). In humid areas, rainfall tends to keep salts and other soluble minerals leached out of soils. In arid areas, naturally saline parent material may still reside in soil (Trout, 2000). Concurrently, water is naturally limited in arid areas so that irrigation is essential for agriculture production and urban landscapes and green spaces. Periodic rainfall or irrigation water can leach salts out of naturally saline soil or parent material, degrading water quality. Poor irrigation water quality can also potentially add considerable salt to otherwise non-saline soil. Saline or brackish water can be used for plant production or use in urban landscapes if soil salinization is minimized through sufficient applied water to flush salts through the profile and out of the root zone.

Soil salinity stresses plants in two ways. Salt toxicity and physiological drought occur when high concentrations of salts in the soil impede water uptake by outcompeting
roots for water. Leaf injury symptoms with chlorosis and margin burn that reduce green leaf area limits photosynthesis and ultimately growth when salts such as Na$^+$ and Cl$^-$ are taken up and accumulate to toxic levels (Bernstein and Hayward, 1958).

Salinity tolerant species can avoid the stress of excess ion several ways: by excluding salt ions from the xylem of the roots (Schubert and Läuchli, 1990) or retaining ions in the root system and allowing remaining ions to be taken up to transpiration stream to the shoots for osmoregulation (Ball, 1988). Most salt tolerant crop species limit salt uptake into the transpiration stream to some degree through membrane-mediated compartmentalization in root vacuoles (Shannon, 1997). Accumulation of ions in the vacuoles of both roots and leaves is clearly one of the most important strategies employed by plant cells against salinity stress by removing potentially toxic ions from the cytoplasm (Mimura et al., 2003). Some species are able to sequestrate ions to special organelles in the leaves such as salt glands or glandular trichomes in order to alleviate ion toxicity (Shimony et al., 1973; Tomaso, 1998).

As salinity increases, soil matric potential becomes more negative. Plants can continue to absorb water only as long as their water potential is lower (more negative) than that of the soil water (Taiz and Zeiger, 2006). Osmotic adjustment (OA) is an accumulation of organic salts to decrease osmotic pressure which in turn decreases total water potential as plants respond to increase soil salinity concentration (Bernstein, 1961). OA is considered an adaptation for surviving rather than growing during stress periods (Taiz and Zeiger, 2006). However, the degree of OA in plants varies among species and genotypes (Gebre et al., 1994; Guicherd et al., 1997; Wang et al., 1996). Most species have the ability to adjust their osmotic potential under stress, such as *Avicennia*
germinans L. (Suárez et al., 1998), Atriplex nummularia L. (Silveira et al., 2009), and Chestnut oak (Quercus prinus L.) (Tschaplinski et al., 1998) while others do not, such as red maple (Acer rubrum L.) and sugar maple (Acer saccharum Marsh.) (Tschaplinski et al., 1998).

Pressure-volume curve (PV-curve) analysis has long been used to explain the relationship between leaf water potential ($\psi_{\text{leaf}}$) and leaf relative water content ($\text{RWC}_{\text{leaf}}$) (Hinckley et al., 1980; Turner, 1981; Tyree and Hammel, 1972). As $\text{RWC}_{\text{leaf}}$ decreases during leaf drying, turgor pressure also decreases, eventually reaching a point at which the cell wall is flaccid and cell water potential equals cell osmotic potential (turgor loss point) (Baltzer et al., 2008). Thus, the PV-curve can be used to estimate osmotic potential at full saturation ($\psi_{\text{sat}}$), osmotic potential at turgor loss point ($\psi_{\text{TLP}}$), relative water content at turgor loss point ($\text{RWC}_{\text{TLP}}$), apoplastic water fraction, and volumetric elastic modulus ($\varepsilon$). $\varepsilon$ is the ratio of the change in cell turgor to that in the relative cell volume (Rada et al., 1989; Saito et al., 2006) which can be calculated from the slope of PV-curve (Steudle and Zimmermann, 1977). Elastic cells (small $\varepsilon$) will sustain a smaller decrease in turgor potential as a given volume of water is lost than will a more rigid cells (large $\varepsilon$) (Joly and Zaerr, 1987). Rigid cells (large $\varepsilon$), in contrast, allow a large difference in water potential between soil and leaves to be produced with relatively little water loss which would, in turn, increase water uptake (Bolaños and Longstreth, 1984).

Salt-induced plant water stress, called physiological drought, occurs when soluble salt levels in the soil solution are high enough to limit water uptake, thereby inducing drought stress (Carrow and Duncan, 1998). Both salt and water stresses lead to cellular dehydration, which causes osmotic stress (Bartels and Sunkar, 2005). Thus, plants may
use common pathways in response to those stresses (Pastori and Foyer, 2002; Tuteja, 2007). Regarding these assumptions, some xeric region plants that have never been found in a saline area may have the ability to be salinity tolerant that can be predicted on a basis of drought tolerant ability. The objectives of this paper are 1) to study the responses to salinity of closely related Acer species differing in native water availability habitats with Eucalyptus species acting as a control species native to saline conditions and 2) to determine the mechanisms to cope with salinity stress in those species.

MATERIALS AND METHODS

Plant materials

Acer grandidentatum Nutt. (bigtooth maple), A. macrophyllum Pursh. (bigleaf maple) and Eucalyptus camaldulensis Dehn. (red gum) were used in the experiment. Two Acer species were selected based on the difference in moisture habitats. They were obtained bare root from local nurseries and transplanted to 1-gallon pots filled with organic medium (Sunshine mix #1, SunGro Horticulture Canada Ltd. and allowed them to grow for 2 months (April-June). Eucalyptus seeds were germinated on germination paper (Seedburo Equipment Company) for 2 months and plants were transferred to 4-L pots (True#1, Polytainer, Nursery Supplies, Inc., Orange, CA) filled with the same organic medium for 2 months. All pots were fertilized with 20 g of a 12.7N – 7.6P – 10.2K controlled-release fertilizer (Osmocote 15-9-12 last for 3–4 months).

Bigtooth maple (Acer grandidentatum Nutt.) is closely related to sugar maple (Acer saccharum Marsh.), but native to the U.S. Intermountain West, extending into west
Texas, on well-drained slopes between 1350 to 2600 m in (Landrum, 1995) and total annual precipitation varies substantially from 258 to 454 mm (Bsoul et al., 2006; Phillips and Ehleringer, 1995). Though bigtooth maple grows best on deep soils, it can do well on shallower soils and drier sites, and is considered to be good candidate low water urban landscapes (Barker, 1977). It tolerates winter temperatures as low as -34°C. (Barker, 1977; Kuhns, 2010; Welsh et al., 1987), and is reported to be drought and salt tolerant (Emad, 2005).

Bigleaf maple (*Acer macrophyllum* Pursh.) is also closely related to sugar maple, but occurs along the Pacific Northwest coast of North America. It grows over a wide range of temperatures (2 to 27 °C), in more mesic conditions than bigtooth maple (annual precipitation 560 to 6600 mm) with an elevation of 915 to 2135 m (Minore and Zasada, 2010). Bigleaf maple has been reported to be moderately drought and heat tolerant, but definitely shade tolerant (Sarr et al., 2011) and appears to be susceptible to salts when growing along road sides that are de-iced during winter time (Dirkse, 2006).

Red gum (*Eucalyptus camaldulensis* Dehnh.) is native to Australia and is the most widely distributed of all Eucalyptus species (Boland et al., 2006). Although mainly riparian, red gum is phreatophytic and is able to extend into floodplains with accessible water tables (Thorburn and Walker, 1994). It thrives in plantations throughout much of the sub-tropical world, including coastal California (Moral and Muller, 1970). It has been reported to be both drought and salinity tolerant (Farrell et al., 1996; Gibson et al., 1994; Grieve et al., 1999; Merchant et al., 2006; Van der Moezel et al., 1988). It could grow at 200 mm rainfall areas during summer and stay visually acceptable under 20% of well-watered plants for 10 weeks (Merchant et al., 2006). A 100% in survival of Eucalyptus
seedlings after 11 weeks under 42 dS m$^{-1}$ of NaCl, MgSO$_4$, and CaCl$_2$ with maintenance of acceptable visual appearance for 3 weeks shows high salinity tolerance in this species (Van der Moezel et al., 1988). Thus, red gum allows comparison in salinity tolerance.

**Salinity treatment application**

These experiments were conducted twice, in fall 2009 (October–November) and summer 2010 (June–September). Salinity treatments were applied using a low volume near-continuous gradient dosing system (Hawks et al., 2009) with 5 treatment levels; 0.4 dS m$^{-1}$ (control treatment with only nutrient solution), 3, 6, 9, 12 dS m$^{-1}$. NaCl and CaCl$_2$·2H$_2$O mixed at ratio of 151 g of NaCl : 809 g of CaCl$_2$ with 1 L of water. A drip irrigation system was assembled in a research greenhouse with two supply laterals. The system begins as irrigation water enters an injector pump which is responsible for injecting nutrient solution (Peter's Excel Multi-Purpose 21-5-20 water soluble fertilizer with 100 ppm N; Everris, Camarillo, CA, USA) into the line. The line then splits, either going into the main nutrient solution delivery lateral or to a second pump. Using the second pump, the water containing nutrient solution is injected with the desired salinity treatment. To control the nutrient and treatment dosages, drip emitters of various flow rates (Rain Bird Corporation, Tucson, AZ) were used.

The total output of all coupled emitters was designed to equal 45.4 L h$^{-1}$ and injection pressure at 20 psi with injection rate of 100:1 (nutrient solution : treatment solution). For example, the control treatment used only a 45.4 L h$^{-1}$ emitter plugged into the nutrient line. For 3 dS m$^{-1}$ treatment, it contained 7.6 L h$^{-1}$ and 3.8 L h$^{-1}$ emitters plugged into the salinity treatment line, and 26.5 L h$^{-1}$ and 7.6 L h$^{-1}$ emitters plugged into
the nutrient line. The system was programmed to water twice a day at 6:00 am and 5:00 pm for 1 min each time to ensure no salt accumulation in the root zone which was indicated by the amount of leachate collected from each pot after irrigation at about 600 ml. EC$_e$ of leachate was measured using a portable conductivity meter (Model Sension5, Hach Company, Loveland, Colorado) every week.

Six plants per species were randomly assigned to each salinity treatment. The salt concentration was increased by 2 dS m$^{-1}$ weekly in 2009 and daily in 2010 until a concentration of 12 dS m$^{-1}$ was obtained. After reaching the desired levels of salinity, plants were allowed to continue growing for a week before data collection commenced.

**Measurements**

Leaf damage measurement

Leaf damage measurement was measured only in 2010. After reaching target EC levels, percent leaf damage was estimated by leaf area reduction using regression technique. Twenty five leaves of two *Acer* species were measured in length and width (cm) and then fed to a portable leaf area meter (Model Li-3000, LI-COR, Lincoln, NE) to measure leaf area. The relationship between length times width and leaf area was established by regression to estimate the leaf area of whole plant. Then, length and width of each leaf in all pots were measured and estimated for leaf area every week for 2 weeks during the EC build up to target levels and subsequent 3 weeks thereafter. Leaf area reduction caused by burning damage was calculated as a percent of the initial leaf area.
Stomatal conductance and leaf water potential measurements

Plant water relations measurements were taken once a week for 3 weeks after target EC levels were obtained with six replications to get an average final value in each species. Only third week data were calculated the statistics on the mean values and exhibited in graphs. Stomatal conductance ($g_s$) was measured with leaf porometer (SC-1 Decagon Devices, Pullman, WA) between 11:00 am to 2:00 pm MDT on a clear day of full sun. A leaf was inserted with the abaxial side (bottom) facing to the open chamber. By an auto mode of the instrument, $g_s$ was measured in 30 s. Leaf water potential ($\psi_{leaf}$) was measured at midday of the same day when $g_s$ was measured. An expanded mature leaf was cut at the petiole and immediately put into the pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Nitrogen gas was slowly applied until water was coming out from the cut end and the pressure was read as $\psi_{leaf}$.

Photosynthesis measurement

Photosynthesis was measured using a portable photosynthesis system with a chlorophyll fluorescence attachment (Model LI-6400, LI-COR, Lincoln, NE) after target EC levels were obtained at week 3 with three replications to get an average final value in each species. An expanded mature leaf of each species per treatment was placed into the fluorescence chamber. The instrument was set with the flow rate at 500 $\mu$mol m$^{-2}$ s$^{-1}$, CO$_2$ at 400 $\mu$mol m$^{-2}$ s$^{-1}$, and light at 1000–1200 $\mu$mol m$^{-2}$ s$^{-1}$ with 10% of blue light. The data were manually logged after photosynthesis, CO$_2$, H$_2$O, and fluorescence were stable.
Pressure-volume curve measurement

Pressure-volume (PV) curves were developing only in July 2010 after all measurements were done as described by Tyree and Hammel (1972) and Hinckley et al. (1980). PV curves were developed only for control and 12 dS m\(^{-1}\) treatments, representing salinity extremes, using plants with three leaves per treatments for each species. Pots were watered and kept in a cool room (4\(^\circ\)C) with no light for 24 hours and wrapped with plastic in order to resaturate leaves. The following day, a fully re-saturated leaf was removed from the plant and weighed to get the fully turgid weight. Then fresh weights were repeated before and after each pressure chamber reading of water potential were made on the leaf. On each occasion, the chamber was pressurized and depressurized very slowly (less than 0.01 MPa s\(^{-1}\)) using a nitrogen gas supplied pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Between readings, the leaf was allowed to transpire freely outside the pressure chamber. After readings were finish, leaves were oven-dried at 80\(^\circ\)C for 48 hours and weighed to determine dry weight.

Osmotic potential at full saturation (\(\psi_{\text{sat}}\)), osmotic potential at turgor loss point (\(\psi_{\text{TLP}}\)), relative water content at turgor loss point (RWC\(_{\text{TLP}}\)), apoplastic fraction and volumetric elastic modulus (\(\Box\)) were calculated from the PV curves (Turner, 1981). Osmotic adjustment was calculated as the different between \(\psi_{\text{sat}}\) of treatment plants and the mean \(\psi_{\text{sat}}\) of control plants (Lazarus et al., 2011).

In the pressure chamber, the turgor pressure is reduced to zero by applying the pressure to the leaves. Once the turgor pressure reaches zero, the volume of the water in the cell is related to applied pressure: \(\frac{1}{F_c} = \frac{V_s - V}{RTN}\)
where $P_c$ is the pressure in the chamber, $V_s$ is the volume of symplastic water in the turgid leaf, $V$ is the volume of the symplastic water expressed, $R$ is the gas constant, $T$ is the Kelvin temperature and $N$ is the moles of solute in the leaf. Thus a plot of $1/P_c$ against $V$ becomes linear when the turgor pressure becomes zero (PV curve). Extrapolation of the straight line $V=0$, gives the $\psi_{sat}$, and the $\psi_{TLP}$ is the point at which the water potential and osmotic potential are equal. Extrapolation of the straight line to $1/P_c = 0$, i.e. infinite pressure, gives the total symplastic water in the leaf ($V_s$).

Total volume of water in the leaf ($V_t$) is determined from the difference between initial turgid weight (TW) and oven-dried weight (DW). Then, the apoplastic water, i.e. water in the cell walls is $V_t - V_s$. It should also be apparent that the relative water content (RWC) is given by: 

$$RWC = \frac{V_t - V}{V_t} \times 100$$

and the relative symplastic water content (RSWC) is given by: 

$$RSWC = \frac{V_s - V}{V_s} \times 100$$

Further, the volumetric modulus of elasticity ($\varepsilon$) is given by: 

$$\varepsilon = \frac{\Delta P}{\Delta RSWC} \times 100$$

**Statistical analysis**

The experiment was a completely randomized 2 factorial design. Species (three levels) and salinity treatments (five levels) with six replications were used in the leachate, leaf damage, $g_s$, and $\psi_{leaf}$ measurements; with three replications used in photosynthesis measurement. Species (three levels) and salinity treatments (two levels; control and 12 dS m$^{-1}$) with three replications were used in PV curve measurement. Treatment effects, species differences, and treatments x species interactions were determined by analysis of variance (ANOVA) according to the GLM procedure of SAS (version 9.0; SAS Institute,
Cary, NC, USA). Mean separation differences were tested with a least significant difference test at a probability level of 0.05. Slopes of stomatal conductance and photosynthesis as percent of control in each species were compared based on quadratic fitting curve using SigmaPlot (version 11.0, Systat Software, Inc., Chicago, IL) with the Snedecor’s $F$ statistic at a probability level of 0.05.

**RESULTS**

**Monitoring salt delivery concentrations**

The leachate EC collected from bare soil pots without plants closely tracked target levels (Table 4-1). Interestingly, the leachate EC$_e$ from *Eucalyptus* containers was significantly higher than target conductivity in every treatment except the control, with an EC consistently about 3 dS m$^{-1}$ higher than the target levels. In contrast, leachate EC$_e$ of both bigleaf and bigtooth maple were close to the target conductivity in every treatment.

**Leaf damage**

Leaf area of Eucalyptus was not affected by any salinity treatment, with no signs of damage on any leaf (Fig. 4-1A). Both *Acer* species, in contrast, showed margin burn as a result of salt toxicity. Leaf damage in bigleaf maple, however, was greater than in bigtooth maple (Fig. 4-1B-C). Therefore, only the two *Acer* species were measured for leaf area reduction. Margin burn was a distinguishing sign of damage in both species, as green leaf area decreased as salinity increased. Bigleaf maple rapidly exhibited differences in leaf area in response to salinity during the first week, whereas it took 3
Table 4-1 Salinity treatments with drip emitter combination and leachate EC\textsubscript{e} collection in 2010.

<table>
<thead>
<tr>
<th>Treatmen nt (dS m\textsuperscript{-1})</th>
<th>Emitter combination\textsuperscript{y}</th>
<th>Leachate EC\textsubscript{e} (dS m\textsuperscript{-1}) ± SE\textsuperscript{x}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nutrient (L h\textsuperscript{-1})</td>
<td>Treatment (L h\textsuperscript{-1})</td>
</tr>
<tr>
<td>Control (0.4)</td>
<td>45.4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>26.5+</td>
<td>7.6+3.</td>
</tr>
<tr>
<td>4</td>
<td>7.6</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>18.9+</td>
<td>18.9+3</td>
</tr>
<tr>
<td>9</td>
<td>3.8</td>
<td>.8</td>
</tr>
<tr>
<td>12</td>
<td>7.6+3</td>
<td>26.5+7</td>
</tr>
<tr>
<td>12</td>
<td>0.8</td>
<td>.6</td>
</tr>
<tr>
<td>12</td>
<td>11.74 ±</td>
<td>15.56 ±</td>
</tr>
<tr>
<td>12</td>
<td>0.14</td>
<td>0.57g</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Salinity solution of 5 different treatments measured in electrical conductivity (dS m\textsuperscript{-1})

\textsuperscript{b} Emitter combination between nutrient solution and salinity treatment solution to obtain final target rate of 45.4 L h\textsuperscript{-1} of 5 different salinity levels

\textsuperscript{c} The leachate collected from each pot with standard error of 6 plants

\textsuperscript{d} The values followed by the same letter in columns and rows are not different at $P < 0.05$.

weeks for bigtooth maple to show differences (Fig. 4-2). In addition, a 50% reduction in green area of plants at 12 dS m\textsuperscript{-1} treatment occurred in the third week in bigleaf maple while it happened in the fourth week in bigtooth maple. At week 5, bigleaf maple leaf area fell to about 10% of control at the highest salinity level while it was about 25% for bigtooth maple.

Stomatal conductance and leaf water potential responses to salinity stress
Stomatal conductance ($g_s$) in Eucalyptus was significantly higher than the *Acer* species in both years ($P < 0.0001$) (Fig. 4-3A-B). The $g_s$ decreased significantly as salinity increased in both *Acer* species. However, there was no difference in the magnitude of $g_s$ between *Acer* species under salinity treatments. According to the relative

![Fig. 4-1](image-url).  Leaf area damage of (A) *Eucalyptus camaldulensis* Dehnh. (Eucalyptus) (B) *Acer macrophyllum* Pursh. (bigleaf maple), and (C) *Acer grandidentatum* Nutt. (bigtooth maple) under 5 different salinity treatments. The same letters are not significantly different at $P < 0.05$ of damage in each species.
Fig. 4-2. Percent leaf area over 5 weeks under 5 different salinity treatments of (A) *Acer macrophyllum* Pursh. (bigleaf maple), and (B) *Acer grandidentatum* Nutt. (bigtooth maple). The same letters are not significantly different at $P < 0.05$ of leaf area in each species.

$g_s$ of treatment to control plants, solution electrical conductivity that caused a 50% reduction in stomatal conductance ($g_{s50}$) was the highest in Eucalyptus (14.4 dS m$^{-1}$) followed by bigtooth maple (9.8 dS m$^{-1}$) and bigleaf maple (3.6 dS m$^{-1}$), respectively, at $P < 0.0001$.

The leaf water potential ($\psi_{leaf}$) at the highest salinity treatment was more negative than the control in Eucalyptus and bigtooth maple, but not in bigleaf maple in 2009 (Fig. 4-3E), while the trend was not clear in 2010, as there was no difference among treatments in all species (Fig. 4-3F). This suggests that salinity had less effect on $\psi_{leaf}$ than on $g_s$. 
However, in comparison among species, bigleaf maple showed the least negative $\psi_{\text{leaf}}$ compared to Eucalyptus and bigtooth.

**Photosynthesis responses to salinity stress**

Photosynthesis in Eucalyptus was greater than in the *Acer* species and it remained unchanged as salinity increased. In contrast photosynthesis of *Acer* species was generally comparable and declined with increasing salinity, similar to $g_s$ (Fig. 4-3C-D). Based on the ratio of treatment to control photosynthesis, the solution electrical conductivity that caused a 50% reduction in photosynthesis ($\text{photo}_{50}$) was the highest in Eucalyptus (15.0 dS m$^{-1}$) ($P < 0.0001$) followed by bigtooth maple (9.1 dS m$^{-1}$) and bigleaf maple (4.7 dS m$^{-1}$), respectively. There was no significant difference between the *Acer* species ($P = 0.109$). Interestingly, bigtooth maple values for all three parameters were different between years, suggesting wide variation within this species.

Under more negative soil water potential as salinity increased, Eucalyptus tended to lower its water potential to be more negative than soil water potential in order to extract water and maintained stomatal conductance (Fig. 4-4) indicating by greater slope in the relationship between ratio of stressed to control plants in stomatal conductance and leaf water potential. The *Acer* species exhibited lower slope than Eucalyptus suggesting less ability to extract water form soil as salinity increased. However, bigtooth maple exhibited the ability to extract water similarly to Eucalyptus at ratio of $g_s$ about 0.6 – 1.0 but the slope declined as stoma closure due to high salinity concentration with no further lowering water potential. Bigleaf maple, in contrast, rapidly decreased in stomatal conductance from ratio of 1.0 to 0.4 without lowering its leaf water potential.
Fig. 4-3. Physiological measurements under different salinity treatments of *Eucalyptus camaldulensis* Dehn. (Eucalyptus), *Acer macrophyllum* Pursh. (bigleaf maple), and *Acer grandidentatum* Nutt. (bigtooth maple) in 2009 and 2010, including stomatal conductance (A-B), photosynthesis (C-D), and leaf water potential (E-F).
Fig. 4-4. The relationship between ratio of stressed to control plants in stomatal conductance and leaf water potential of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple). Data points were fitted with linear regression.

**Pressure-volume curve**

Osmotic potential at full turgor ($\psi_{\text{sat}}$) of salinity treatment plants was greater (more negative) than that of control plants only in Eucalyptus (Fig. 4-5A). In contrast, $\psi_{\text{sat}}$ of the *Acer* species was more (less negative) in salinity treatment plants than in control plants (Fig. 4-5A). Osmotic potential at turgor loss points ($\psi_{\text{TLP}}$) showed the same trend as osmotic potential at full turgor (data not shown). Relative water content at turgor loss point (RWC$_{\text{TLP}}$) was not significantly different among species and treatments and ranged between 0.90–0.95 (data not shown).
Fig. 4-5. Pressure-volume curve analysis of *Eucalyptus camaldulensis* Dehnh. (Eucalyptus), *Acer macrophyllum* Pursh. (bigleaf maple), and *Acer grandidentatum* Nutt. (bigtooth maple) under control and 12 dS m\(^{-1}\) treatments in (A) osmotic potential at full turgor, (B) osmotic adjustment, (C) Apoplastic water fraction, and (D) Modulus elasticity (\(\varepsilon\)). The same letters are not significantly different at \(P < 0.05\) of leaf area in each species.

There was an evidence showing that Eucalyptus had the ability to osmotically adjust (OA), as indicated by the difference between \(\psi_{\text{sat}}\) and \(\psi_{\text{TLP}}\) at approximately 0.8 MPa (Fig. 4-5B). In contrast, the *Acer* species clearly showed no OA under salinity stress (Fig. 4-5B). The percent apoplastic fraction (% Apoplastic) was different between treatment and control plants only in bigtooth maple (Fig. 4-5C) as the plants receiving high salt concentration had higher % Apoplastic water than control plants. The volumetric elastic modulus (\(\varepsilon\)) showed two different trends, which was higher in
treatment plants than in control plants in Eucalyptus, but lower in the Acer species (Fig. 4-5D). However, bigleaf maple showed no statistical difference in □ between treatments.

**DISCUSSION**

Eucalyptus showed the greatest salinity tolerance among all species as might be expected from other research (Farrell et al., 1996; Grieve et al., 1999). While not as great as Eucalyptus, bigtooth maple exhibited greater salinity tolerance than bigleaf maple which may be linked to its adaptation to a lower rainfall habitat (Barker, 1977). Thus, our results suggest that there is cross-tolerance between drought and salinity (Farooq and Azam, 2001; Pastori and Foyer, 2002; Tuteja, 2007) as bigtooth maple from drier habitats exhibited greater salinity tolerance while bigleaf maple was drought intolerant and turned out to be susceptible to salinity.

Eucalyptus utilized a variety of strategies to cope with salinity stress. One of the most important mechanisms to prevent tissue damage is salt exclusion at root level (Bernstein and Hayward, 1958; Munns and Tester, 2008). Our results confirmed that salt exclusion by the higher leachate EC$_e$ found in every salinity treatment (Table 4-1). Similar elevated EC$_e$ have been also reported for *E. camaldulensis*, also a salt excluding species (Farrell et al., 1996; Nasim et al., 2009; Van der Moezel et al., 1988). The sodium exclusion mechanism is believed to involve reabsorption from the xylem and retention in the proximal root and lower stem (Walker, 1986). Most of the Na$^+$ that enters root cells in the outer part of the root is likely pumped out via plasma membrane Na$^+$/H$^+$ antiporters (Munns and Tester, 2008). Eucalyptus has also been reported to possess the ability to
sequester larger amounts of salt into senescing lower leaves or dilute the volume of salts over a greater volume of leaf material (Farrell et al., 1996). Unlike Eucalyptus, both Acer species did not exhibit the ability to exclude salts at root levels to prevent ion uptake from saline substrate (Table 4-1).

Eucalyptus also exhibited the ability to adjust osmotically (Fig. 4-5B) to maintain water uptake from saline soil (Verslues et al., 2006). Osmotic adjustment (OA) has been cited as a tolerance mechanism in both salt and drought stress (Gupta and Berkowitz, 1987; Hinckley et al., 1980; Lemcoff et al., 2002; Munns, 1988; Suárez et al., 1998). OA involves physiological maintenance costs associated with synthesis of solutes, ion transport, and repair of cell structures (Kozlowski, 1997). Numerous Eucalyptus species from arid regions grow well under moderately saline conditions and utilize OA by accumulating organic acids to osmotically significant concentrations (Adams et al., 2005) in order to lower the water potential in their cells.

In addition, Eucalyptus showed high \( \psi \) (rigid cell wall) under high salinity in this study which allowed \( \psi_{\text{leaf}} \) to drop faster for the same loss of water (Fig. 4-4). Thus, Eucalyptus under salinity stress was able to maintain water flow from the soil through a greater water potential gradient for a smaller loss of water (Lenz et al., 2006). Integrating the salt exclusion and water extraction mechanisms. OA allowed cells to maintain turgor pressure and continue to grow normally under salinity stress. Integrating the above mechanisms together, Eucalyptus exhibited salinity tolerance resulting in maintaining visual appearance, \( g_s \), and photosynthesis unchanged as salinity increased (Figs. 4-1 and 4-3).
Both *Acer* species in this study showed substantially lower salinity tolerance than *Eucalyptus* as indicated by visual appearance, as both showed leaf margin burn damage (Fig. 4-1B-C). However, bigtooth maple exhibited higher salinity tolerance than bigleaf maple based on all criteria. Leaf margin burn in bigtooth leaves appeared later, in week 3–4, and only under the highest salinity concentrations (9 and 12 dS m\(^{-1}\)) (Fig. 4-2B). These results were similar to the studies of Emad (2005) indicating that visual appearance showed no significant differences among treatments at the highest salt concentration (10 dS m\(^{-1}\)) in bigtooth maple from different sources. Bigtooth maple was documented to have a slightly better appearance under salt irrigation water (2 dS m\(^{-1}\)) than deionized water and was recommended to plant in landscapes where water is saline (Hatter and Morgan, 1992).

Even though bigtooth maple have no OA mechanisms (Fig. 4-5A-B), it still maintained turgor pressure under high salinity concentration via elastic cell walls (Fig. 4-5D). When cells lose water, they decrease in volume until turgor is completely lost. Plants with highly elastic walls (low $\varepsilon$) have more flexibility maintaining symplastic volume at reduced turgor pressures. Thus, stressed bigtooth maple plants showed lower $\varepsilon$ than control plants in this study suggesting that they maintained more water at full turgor, hence, their volume can decrease more before turgor-loss point is reached (Lambers et al., 1998). As a result, bigtooth elastic cells could maintain their turgor pressure under salinity stress (Chai et al., 2010; Lenz et al., 2006), resulting in continuing stomatal opening and photosynthesis at intermediate salinity levels (Fig. 4-3), helping the plant to maintain integrity and appearance.
Bigleaf maple was the most sensitive to salinity in this study. This could be linked to its mesic habitat. Even though bigleaf maple grows over a wide range of moisture condition, it is usually found on riparian sites (Minore and Zasada, 2010) from British Columbia-Canada to northern California-U.S. Thus, it may likely use substantial water via transpiration due to the large hydraulic and conduit diameter (McCulloh et al., 2010; Waring et al., 1976). Since our results indicated that bigleaf maple had no salt exclusion mechanism (Table 4-1), most ions from the saline solution would be taken up along with transpiration mechanism causing leaf injury by ion toxicity (Figs. 4-1 and 4-2A). High salt concentration in the soil could directly damage root tissues causing top dieback as the plant was unable to sustain the canopy due to a progressive root loss (Guttay, 1976). Bigleaf maple plants along roadside have been reported to show browned, curled leaf margin due to anti-icing salt (calcium chloride) use causing high root zone Cl⁻ concentration throughout a 7-mile stretch of highway in Washington state in the Pacific Northwest (Dirkse, 2006).

Stomatal responses are undoubtedly affected by osmotic impact of root zone salt concentrations. Species that fail to exclude salts, such as bigleaf maple, rapidly manifest toxic impact within days (Munns, 2002). Salts may build up in the apoplast and dehydrate the cells, and they may build up in the cytoplasm and inhibit enzymes involved in carbohydrate metabolism, or they may build up in the chloroplast and exert a direct toxic effect on photosynthesis processes, causing reduction in photosynthesis (Fig. 4-3) (Munns and Tester, 2008).

Osmotic potential at full turgor in bigleaf maple was lower in control (more negative) than in the salinity treatment (Fig. 4-5A) but not statistically different.
suggesting no OA in this species. This could happen because turgor pressure reached the flaccid point fast due to tissue damage by toxic salts. Thus, cells had no energy to accumulate solute in order to lower their osmotic potential (Lemcoff et al., 2002). In other words, cytoplasm tissues were dead before they could adjust the osmotic potential. In conclusions, bigleaf maple did not show OA (Fig. 4-5A-B), and showed no difference between control and salinity treatments in apoplastic water fraction and suggesting that it did not have any cell-level tolerance mechanisms to cope with salinity stress. As a result, stressed plants exhibited the obvious sign of leaf injury at the first week of experiment (Figs. 4-1B and 4-2A).

In conclusions, plants are genetically and habitually different in responses and mechanisms to cope with salinity stress. Based on this study, *Eucalyptus camaldulensis* showed the most salinity tolerant species by having salt exclusion mechanism at root level and OA under salinity stress resulting in maintenance of visual appearance, stomatal opening, and photosynthesis rate, same as control plants. In contrast, both *Acer* species had no ability to exclude salts at root level. However, Bigtooth maple showed the ability to maintain turgor at lower relative water content via increasing cell wall elasticity and high water uptake under low and medium salinity stress. Bigleaf maple was the least salinity tolerance species in this study due to no ability to either avoid or tolerate salinity stress. These data suggest that other drought tolerance tree species are likely to exhibit salinity tolerance, which is very useful when planting in urban landscapes where brackish or very slightly saline water may be used.
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Drought stress is common in arid and semi-arid areas causing lower water potential in soils, which makes it hard for plants to take up water. Osmotic stress initially occurs in both drought and salinity stress. Thus, turfgrasses may respond and have similar mechanisms to cope with both stresses. The objectives of this study were to study the morphological and physiological responses of known salinity tolerant entries, including three Kentucky bluegrass (KBG) varieties or populations (*Poa pratensis* L.)—Tolerant KBG (PI372742), Moderate KBG (‘Midnight’ KBG), and Susceptible KBG (PI368233)—and one tall fescue variety (*Schedonorus phoenix* (Scop.) Holub)—‘Matador’ TF—under drought stress; and to determine the common mechanisms to cope with drought stress for those differing salinity tolerant entries. Plants were subjected to a well-watered treatment of 130% of evapotranspiration (ET), and dry down treatments of 90% and 75% of ET in 2010 and 2011, respectively. ET was measured from water loss from containers each day and volumetric soil water content was calculated based on water left in the containers. Turf quality (TQ); Stomatal shape, size, and density; stomatal conductance ($g_s$); leaf water potential ($\psi_{leaf}$); photosynthesis; water use efficiency (WUE) were measured. The results suggest that all entries were equally drought tolerant but

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1 Coauthored by Nisa Leksungnoen, Paul G. Johnson, and Roger K. Kjelgren
differed in mechanisms to cope with the drought. ‘Matador’ TF consumed rapid water
due to a deep root system and extracted water by lowering leaf water potential to
compensate with high ET. Moderate KBG tended to conserve water with low ET and
consumed soil water slowly by not allowing more negative leaf water potential. Tolerant
KBG and Susceptible KBG were between ‘Matador’ TF and Moderate KBG in responses
and mechanisms. These two entries tended to allow more negative water potential to
extract water from soil to maintain their metabolisms during drought. Salinity tolerant
species of ‘Matador’ TF, Tolerant KBG, and Moderate KBG were also drought tolerant
as expected. In contrast the salinity intolerant Susceptible KBG was also concluded to be
drought tolerant.

INTRODUCTION

Global aridity is predicted to be widespread in coming decades due to climate
change and drought stress, similar to what occurs now in semi-arid and arid regions (Dai,
2011). Large-scale drought mainly causes reductions in annual net primary production
(Zhao and Running, 2010). Temperatures are projected to rise 21th century by 2 – 11.5°C
depent upon the greenhouse gas emission scenario (IPCC, 2007). Urban landscape cool-
season turfgrasses are not immune to the warming and possibly are more prone to heating
and drought due to the shallow, low-irrigated soil with confined rooting volumes to
provide enough water (Kjelgren et al., 2004)

Drought influences plants differently over time. In the first minute, cells lose
water and shrink. Over hour, cells regain their original volume but cell elongation rates
are reduced, leading to lower rates of leaf and root growth. Over, days, changes in cell elongation and cell division occur, leading to slower leaf appearance and smaller final size, with leaf growth usually more affected than root growth. After weeks, lateral shoots have been inhibited. After months, the flowering time is altered and the seed production is reduced (Munns, 2002). Thus, plants that are subjected to drought for a long period, or are native to the xeric region are likely to have slow growth rates (Bsoul et al., 2006).

Drought limits cell expansion and growth can be greatly reduced correspondingly (Fry and Huang, 2004), due to a decrease in the availability of soil water which can be quantified as a decrease in water potential (Verslues et al., 2006). The drier the soil becomes, the lower soil water potential (more negative). Stomatal aperture is positively related to turgor pressure of the guard cells that have pores through which water moves in or out, due to changes in guard cell water potential gradient (Buckley, 2005). When drought occurs, water potential in guard cells is less negative and water moves out of guard cells, resulting in stomata pore closure. It has long been known that stomata regulate CO$_2$ uptake and water vapor loss; thus closing stomata can decrease both photosynthesis and transpiration (Fry and Huang, 2004; Zieger, 1983).

Turfgrasses grown in water-limiting environments utilize various adaptive mechanisms including drought avoidance and drought tolerance. Plants may exhibit more than one strategy to cope with drought stress (McCann and Huang, 2007). Drought avoidance is the ability of plants to postpone tissue dehydration by reducing transpiration and/or maintaining water uptake (Fry and Huang, 1999; McCann and Huang, 2007). Tall fescue (TF) is well known to have extensive, deep root systems to extract water from soil.
(Ervin and Koski, 1998; Huang and Gao, 2001); therefore it is classified as a drought
avoidant species.

Drought tolerant species experience drought stress and quiescence (Fry and
Huang, 2004; Volaire and Norton, 2006). In many cases, grasses go dormant and leaves
may desiccate and die, but the crowns survive and the plant recovers when adequate
rainfall and good growing conditions return (Laude, 1953) as show by KBG in the field
(Ervin and Koski, 1998; See Chapter 2). Another important mechanism is the ability to
maintain adequate turgor pressure during drought stress through osmotic adjustment
(Huang and Fry, 1999) and/or changes in cell wall elasticity (Verslues et al., 2006) which
allows plants to take up water at lower soil water potentials (Perdomo et al., 1996; White
et al., 1992). Osmotic adjustment under stress conditions has been reported to occur in
both TF (Qian and Fry, 1997; West et al., 1990; White et al., 1992) and KBG (Jiang and
Huang, 2001; Perdomo et al., 1996).

However, plants may express similar signals when they are exposed to drought
and/or salinity stresses (Tuteja, 2007) because high salt concentrations induce the same
osmotic stress effects on plants as drought (Carrow and Duncan, 1998). Both stresses
show a high degree of similarity with respect to physiological, biochemical, molecular
and genetic effects (Sairam and Tyagi, 2004). The major difference between these to
stresses is the total amount of water available at root zone. In the case of salinity, water
surrounds at root zone with a more negative water potential due to high salt
concentration, while there is not enough water at root zone in the case of drought stress
(Taiz and Zeiger, 2006).
Urban landscape turfgrasses are often subjected to poor irrigation water which contains high salt concentrations (Marcum, 2006) during summer time. Therefore, we hypothesized that salinity tolerant turfgrasses may have cross-tolerance with drought tolerant ability (Pastori and Foyer, 2002). Thus, it should not be surprising if salinity tolerant species could also be drought tolerant species or vice versa, and have similar mechanisms to cope with those stresses (Ashraf and O’Leary, 1996; Farooq and Azam, 2001; Glenn et al., 2009; Trivedi et al., 1991). The objectives of this study were 1) to study the morphological and physiological responses of known salinity tolerant entries under drought stress and 2) to determine the mechanisms of those entries to cope with drought stress.

MATERIALS AND METHODS

The experiments described below were conducted twice, once in 2010 and in 2011. Differences between the two runs of the experiment are highlighted where necessary.

Three KBG varieties or populations (Poa pratensis L.) and one TF variety (Schedonorus phoenix (Scop.) Holub) were chosen for this study. The variety ‘Midnight’ KBG was identified as moderately salinity tolerant (Robins et al., 2009) and the National Plant Germplasm accessions ‘S-107’ (PI372742) and ‘67-126’ (PI368233) were identified as salinity tolerant and intolerant respectively (Robins et al., 2009). ‘Matador’ TF was also used as a drought and salinity tolerant check. Therefore, Susceptible KBG,
Tolerant KBG, Moderate KBG, and ‘Matador’ TF will refer to accession PI368233, accession PI372742, ‘Midnight’ KBG, and ‘Matador’ TF, respectively.

**Volumetric Water Content**

Plants were grown under normal conditions at 25°C/15 °C day/night temperature with natural light in the greenhouse in 3.8- by 21-cm with 1.5-cm depth containers (Ray Leach Cone-tainers, Stuewe and Sons, Corvallis, OR) for 5 months (March–July). Then, seven plants were transferred to 10-cm inner diameter with 23-cm height PVC with mesh at the bottom of the containers. Each container was filled with Sphagnum Peat Moss medium (Sunshine mix #1, SunGro Horticulture Canada Ltd.).

Total volume of each container was 1806 cm³ and the weight of each container (W_C) was measured. The containers were filled with dry medium (217 g) and tapped against the table to allow the media to settle in order to bring the bulk density to 0.15 m³ m⁻³. At this point, the container plus dry medium (W_CS) was weighed. Then, plants (W_P) were weighed and carefully inserted into containers without losing any medium and minimizing medium disturbance.

The water content was obtained by gravimetric method in which the whole container was weighed to obtain the total weight of container plus dry medium, and plant (W_CSP). Then, the bottom of each container was immersed under water over night and weighed at 8:00 h of the next day after water was drained for 2 hours in order to get container capacity weight (W_FC). In summary, dry medium weight (M_S), volume of dry medium (V_T), plant wet weight (W_P), and weight of added water (W_W) were obtained.
Two treatments were applied; well-watered and dry down treatments. Containers were weighed at 8:00 h every day to obtain weight loss from ET of the day before. For well-watered treatment, 130% of water loss was added to the containers to maintain well-watered functions. For dry down treatment, 90% of water loss was added to the containers. In 2010, 75% of water loss was added each day in 2011.

Volumetric water content (VWC; m$^3$ m$^{-3}$) was derived from the volume of water ($V_w$) calculated from the mass of water loss ($W_w$ = Weight of water loss = volume of water remained after ET each day) divided by the total volume of container ($V_T$=1806 m$^3$). VWC was normalized by dividing dry down VWC by well-watered VWC. Then, normalized VWC was plotted against time.

**Morphological measurements**

Turf quality (TQ)

Each container was photographed every week using a white background in order to observe leaf injury resulting from drought stress. Turf quality was rated from 0 to 5, where 0 represented the completely brown canopy and 5 represented the healthy green canopy. Normalized TQ was calculated followed the description in normalized VWC, then plotted against time and VWC.

Stomatal shape, size, and density

Only well-watered plants were used in this study. Leaf punches were collected using a paper hole puncher and directly fixed in formalin-aceto-alcohol (FAA) solution.
The fixed leaf tissue was subjected to critical point drying using Samdri-PVT-3D (Tousimis, Rockville, MD). Three fixed leaf tissues were used to measure stomatal shape, size, and density on both adaxial (upper) and abaxial (lower) surfaces under a scanning electron microscopy (SEM) (Hitachi S4000, Pleasanton, CA).

**Physiological measurements**

Evapotranspiration (ET)

Evapotranspiration rate was determined by the water loss from container each day and plotted against VWC.

Stomatal conductance (gₚ)

Stomatal conductance (gₛ) was measured with a leaf porometer (SC-1 Decagon Devices, Pullman, WA) between 11:00–13:00 h MDT every day. Intact four to five adaxial side (top) blades of each entry per treatment were placed in chamber. While in automatic mode, gₛ was measured in 30 s. Seven replications of the measurements were made with normalized gₛ plotted against VWC.

Leaf water potential ($\psi_{leaf}$)

Five stems of each entry per treatment were used to measure leaf water potential ($\psi_{leaf}$) between 13:00–14:00 h MDT every day. Stems were cut slightly above the root and placed in the pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Nitrogen gas was slowly applied to increase the chamber’s
atmospheric pressure until water appeared at the cut end of the stem. The pressure reading was then taken and used as $\psi_{\text{leaf}}$ and plotted against VWC.

Photosynthesis rate and water use efficiency (WUE)

Photosynthesis was measured every week using a portable photosynthesis system (Model LI-6400, LI-COR, Lincoln, NE). One blade of each entry per treatment was placed into the fluorescence chamber. The instrument was set with the flow rate at 500 $\mu$mol m$^{-2}$ s$^{-1}$, $\text{CO}_2$ at 400 $\mu$mol m$^{-2}$ s$^{-1}$, and light intensity at 1200 - 1500 $\mu$mol m$^{-2}$ s$^{-1}$ with 10% of blue light. The data were manually logged after photosynthesis, $\text{CO}_2$, $\text{H}_2\text{O}$, and fluorescence were stable. Three replications were measured.

Water use efficiency (WUE) was calculated using equation;

$$\text{WUE} = \frac{\text{Photosynthesis (}$\mu$\text{mol}$\cdot$ m$^{-2}$s$^{-1}$)}{\text{Evapotranspiration rate (mol}$\cdot$m$^{-2}$s$^{-1}$)} \times 100$$

where photosynthesis was obtained from Li-6400 measurement and evapotranspiration rate was calculated from water loss from container each day in grams per day and converted to mol per meter square per second based on the surface area of container (radius = 0.0508 m). Then normalized photosynthesis and WUE were plotted against VWC.
Statistical analysis

The experiment was a completely randomized design with two factors; irrigation regimes (two levels) and grass entries (four levels) with seven replications for TQ, ET, g$_s$, and $\psi_{\text{leaf}}$ measurements; and with three replications for stomatal density, photosynthesis, and WUE measurements. Treatment effects, entry differences, and treatments x entry interactions were determined by analysis of variance (ANOVA) according to the GLM procedure by SAS (version 9.0; SAS Institute, Cary, NC). Mean differences were tested with the least significant difference test at a probability level of 0.05. Regression lines of each relationship mentioned in the measurement section were obtained from SigmaPlot program (version 11.0; Systat Software, Inc., San Jose, CA). Slopes of the treatment lines over time were compared using sum of square calculation from linear and non-linear fitting lines by SigmaPlot with the Snedecor’s $F$ statistic at a probability level of 0.05.

RESULTS

Volumetric water content

The volumetric water content (VWC) of well-watered containers was maintained between 0.65–0.70 m$^3$ m$^{-3}$. For dry down treatment, VWC of containers gradually decreased over time (Fig. 5-1). The pooled data over 2 years were presented based on the normalized VWC values of each year. VWC of the TF pots dropped the fastest, followed by Susceptible KBG and Tolerant KBG which dropped at the similar rate ($P = 0.155$). VWC of the Moderate KBG pots dropped the slowest over time.
Fig. 5-1. Normalized volumetric water content of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with quadratic equations with coefficient of determination ($R^2$). The same letters in the parenthesis after each species symbol are not significantly different at $P < 0.05$ in slope of reduction in volumetric water content.
Morphological measurements

Turf quality

Turf quality (TQ) of dry down plants relative to control plants decreased over time (Fig. 5-2A) with TF decreasing faster than in KBG entries ($P < 0.0001$). TQ of dry down TF was significantly lower than that of control plants by week 5 while TQ of dry down KBG entries remained unchanged until week 7 when differences in visual quality between treatments occurred. It took 6 weeks for TF to reach a 50% reduction in TQ compared to the control (well-watered) treatments, while it took 8 weeks for KBG to reach the same point (Fig. 5-2A).

Larger percentages for TQ indicate ability to maintain quality at lower VWCs (Fig. 5-2B). This analysis exhibited no difference among entries in TQ ($P = 0.941$) over VWC ranges suggesting that all entries maintained equal TQ over the dry down period and dropped TQ at a similar rate as VWC decreased.

Stomatal shape, size, and density

The species and entries studied here have the greatest number of stomata on the adaxial (upper side) of the leaf. Stomata were rectangular in shape with a length of 30-33 μm and sunken below the epidermis cells. Density of stomata per mm² was different among KBG entries with Tolerant KBG as the densest at 180 stomata mm⁻² followed by Moderate KBG and Susceptible KBT at 120 and 94 stomata mm⁻² respectively (Fig. 5-3B-D). Unfortunately, due to the uneven distribution of stomata in TF on the upper side of the leaf, no density measurement was possible. Stomata in TF on the lower side of the leaf.
leaf (abaxial) had a density of 52 stomata mm\(^{-2}\) but this is a much lower density than visually observed on the upper side.

Fig. 5-2. Normalized turf quality against (A) time and (B) volumetric water content of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with cubic equations with coefficient of determination (R\(^2\)). The same letters in the parenthesis after each species symbol are not significantly different at P < 0.05 in slope of turf quality reduction over time. Slope of turf quality reduction rate over volumetric water content was not different among entries at P < 0.05.
**Fig. 5-3.** Abaxial cross section of leaves observed under scanning electron microscope (SEM) of (A) ‘Matador’ tall fescue (‘Matador’ TF), (B) Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), (C) ‘Midnight’ Kentucky bluegrass (Moderate KBG), and (D) Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG) with shape and length in µm.

**Physiological measurements**

**Evapotranspiration**

The evapotranspiration rate (ET) of the grasses decreased as VWC of the soil decreased (Fig. 5-4). TF showed the highest ET followed by Susceptible KBG and Tolerant KBG which were similar, with Moderate KBG exhibiting the lowest ET.
Fig. 5-4. Normalized evapotranspiration against volumetric water content of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with cubic equations with coefficient of determination ($R^2$). The same letters in the parenthesis after each species symbol are not significantly different at $P < 0.05$ in slope of evapotranspiration rate as volumetric water decreased.
Stomatal conductance

Stomatal conductance ($g_s$) of dry down plants relative to control plants decreased as VWC decreased (Fig. 5-5). The cubic slope comparison among entries indicated that Tolerant KBG had the same $g_s$ rate as Moderate KBG ($P = 0.157$), but higher than TF ($P = 0.002$) and Susceptible KBG ($P = 0.007$) that exhibited the least $g_s$ rate over VWC range.

VWC that caused a 50% reduction in $g_s$ of TF, Tolerant KBG, Moderate KBG, and Susceptible KBG was 0.051, 0.031, 0.044, and 0.036 $m^3$ $m^{-3}$ respectively. Based on this criterion, Tolerant KBG and Susceptible KBG were the most drought tolerant entries due to their ability to maintain higher $g_s$ at the lowest VWC levels. In addition, the day that Tolerant KBG $g_s$ started showing a significant difference between dry down and well-watered treatments was the longest (54 days), followed by Susceptible KBG (48 days), Moderate KBG (44 days), and TF (37 days) (data not shown).

Leaf Water potential

Leaf water potential ($\psi_{leaf}$) gradually decreased (more negative) as VWC decreased (Fig. 5-6). Moderate KBG showed the highest $\psi_{leaf}$ (less negative) as VWC decreased ($P = <0.0001$) while the other 3 entries showed similar $\psi_{leaf}$ under the VWC range. In addition, Moderate KBG took the longest (51 days) to show a difference in xylem water potential between followed by Tolerant KBG (47 days), Susceptible KBG (44 days), and TF (33 days) (data not shown).
Fig. 5-5. Normalized stomatal conductance against volumetric water content of
‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742)
(Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky
bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2
years and fitted with cubic equations with coefficient of determination ($R^2$). The same
letters in the parenthesis after each species symbol are not significantly different at $P < 0.05$ in slope of stomatal conductance.
Fig. 5-6. Leaf water potential of dry down treatment against volumetric water content of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with cubic equations with coefficient of determination ($R^2$). The same letters in the parenthesis after each species symbol are not significantly different at $P < 0.05$ in slope of leaf water potential.

Drought induced decreases in both $g_s$ and $\psi_{\text{leaf}}$. The linear relationship between the ratio of dry down treatment relative to control of $g_s$ and $\psi_{\text{leaf}}$ (Fig. 5-7) was used to explain how stomata controlled $\psi_{\text{leaf}}$ under stress. The angle of the slopes indicates that as stomata closed; the $\psi_{\text{leaf}}$ also dropped, enabling the plant to continue extracting water from the soil medium. The greater the slope, the greater the ability of plants to withdraw
water from media at the same level of stomatal conductance. Tolerant KBG had the greatest slope ($P = 0.005$) while Moderate KBG exhibited the lowest slope indicating that dry down roots had less ability to extract water from dry soil compared to Tolerant KBG. TF and Susceptible KBG were intermediary between the former two species.

**Fig. 5-7.** The ratio of dry down treatment to control between stomatal conductance and leaf water potential of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with linear equations with coefficient of determination ($R^2$). The same letters in the parenthesis after each species symbol are not significantly different at $P < 0.05$ in slope of the relationship.
Photosynthesis rate and water use efficiency

Photosynthesis rate and water use efficiency (WUE) of plants in the dry-down treatments relative to control plants decreased as VWC decreased but with no difference in slope among entries (Fig. 5-8). WUE of all entries was higher than 100% in much of the VWC range, indicating that plants in drier soil conditions used less water than well-watered plants, but showed an equal photosynthesis output (Fig. 5-8B).

Fig. 5-8. Normalized photosynthesis (A) and water use efficiency (B) against volumetric water content of ‘Matador’ tall fescue (‘Matador’ TF), Kentucky bluegrass accessions ‘S-107’ (PI372742) (Tolerant KBG), ‘Midnight’ Kentucky bluegrass (Moderate KBG), and Kentucky bluegrass accessions ‘67-126’ (PI368233) (Susceptible KBG). Data were pooled over 2 years and fitted with quadratic equations with coefficient of determination ($R^2$). There was no significant difference at $P < 0.05$ in slope of both photosynthesis and water use efficiency.


**DISCUSSION**

All entries in this study exhibited equal drought tolerance in responses, especially in visual appearance (Fig. 5-2), but differed in mechanisms to cope with the drought. In addition, drought tolerance may be unrelated to salinity tolerance since Susceptible KBG, although salt susceptible, showed reasonable tolerance to drought. This lack of relationship was also reported in goatgrass (Farooq and Azam, 2001) and in lupin (Yu and Rengel, 1999). However, the salt tolerant entries—‘Matador’ TF, Tolerant KBG, and Moderate KBG—also exhibited drought tolerance as hypothesized, as observed in wheat (Shaheen and Hood-Nowotny, 2005; Trivedi et al., 1991), quaibush (Glenn et al., 2009), and sunflower (Ashraf and O’Leary, 1996). Therefore drought tolerance does not always translate into salt tolerance. Salt stress may impose different levels of stress, over different time periods, and may also involve ion toxicity (Munns, 2002). Therefore, the responses of plants to salinity and drought stresses may overlap but be not entirely the same due to the different level of stress, time periods, and toxicity caused by salt concentration only.

All entries exhibited acceptable TQ (Fig. 5-2A) even at low VWC (0.2 m$^3$ m$^{-3}$). TQ was reduced by 50% when VWC was about 0.1 m$^3$ m$^{-3}$ (Fig. 5-2B) indicating that all entries in this study were able to maintain visual quality under severe drought stress. These results contrast with that of Fu et al. (2004) where TQ in KBG dropped down below acceptable quality even at the 100% ET while TF maintained acceptable TQ under 60% ET.
‘Matador’ TF had the highest transpiration rate (Fig. 5-4) which in turn depleted soil water the quickest (Fig. 5-1) resulting in the greatest drop in TQ (Fig. 5-2A) but not significantly different than KBG in terms of TQ over VWC (Fig. 5-2B). These trends can be explained by the normally deep root system of TF, which could not be achieved in the 23 cm tubes, and normally would be deeper than KBG (Ervin and Koski, 1998; Sheffer et al., 1987) which allows TF to maintain ET levels as the soil dries (Carrow, 1996; Huang and Fu, 2000; Karcher et al., 2008). High ET in TF was associated with low canopy resistance resulting from low shoot densities, high leaf surface areas, and rapid rates of vertical leaf extension (Huang and Fry, 1999; Kim and Beard, 1988).

TF, however, has been considered to have low water use efficiency (Zhao et al., 1994) due to high ET, 9% higher than KBG in a recent field study (See Chapter 2) with a photosynthesis rate similar to KBG (Fig. 5-8A). In contrast, the Fu et al. (2007) study did not show differences in WUE. In this study, WUE in ‘Matador’ TF was not significantly different from KBG (Fig. 5-8B) due to large amounts of variation in photosynthesis data and TF’s truncated root depth.

‘Matador’ TF kept lowering its \( \psi_{\text{leaf}} \) to extract more water while soil VWC was decreasing (Figs. 5-6 and 5-7), similar to a recent field study (See Chapter 2). In addition, stomata had less control of \( \psi_{\text{leaf}} \) since \( \psi_{\text{leaf}} \) continued to decrease while \( g_s \) was already closed (Schultz, 2003). This could be described as a “use-it-or-lose-it” behavior in terms of water use. This strategy might be considered inappropriate for low water landscapes under minimal or no irrigation (Kjelgren et al., 2009) because if TF cannot rely on a deep root system to extract water due to shallow soil or a restricted rootzone, it is not likely to
perform well when water is limited (See Chapter 2).

Moderate KBG (‘Midnight’ KBG) has been reported to have low ET rates (Ebdon and Petrovic, 1998; Ebdon et al., 1998; Fu et al., 2007) which agrees with our study (Fig. 5-4) as it exhibited the lowest ET among all entries. As a result, its WUE was the highest (Fig. 5-8B). The $g_s$ could be used to explain the WUE because its $g_s$ was relatively high (Fig. 5-5) indicating more opportunity to exchange CO$_2$ via stomata (Zeiger, 1983) which in turn resulted in higher photosynthesis rates (Fig. 5-8A) along with the low ET (Fig. 5-4).

Interestingly, Moderate KBG opened more stomata, as measured by stomatal conductance, creating a greater photosynthate potential but at a lower ET rate. This potentially makes this variety a superior entry under drought stress (Fry, 2000). Moderate KBG tended to maintain its $\psi_{leaf}$ (Fig. 5-6) in order to conserve water from ET (Fry, 2000). Even though this entry has been reported to exhibit osmotic adjustment to lower its water potential under summer stress (Perdomo et al., 1996), it seemed not to have used that mechanism under this situation. This particular drought tolerant mechanism could be described as a “save-it-for-a-rainy-day” behavior (Kjelgren et al., 2009) in which stomata seem to control and prevent $\psi_{leaf}$ to be more negative (Figs. 5-5 and 5-6). This evidence clearly showed in Fig. 5-8 that the slope of the ratio between $g_s$ and $\psi_{leaf}$ of Moderate KBG was the lowest.

Tolerant KBG and Susceptible KBG seemed to be intermediate between ‘Matador’ TF and Moderate KBG in responses and mechanisms to cope with drought in this study. However, considering the ratio of $g_s$ and $\psi_{leaf}$ (Fig. 5-7), Tolerant KBG
responded more like ‘Matador’ TF in terms of aggressive usage of water while Susceptible KBG was more like Moderate KBG where gs appeared to control ψleaf (Figs. 5-7).

All entries possessed different mechanisms to cope with drought which involved an adaptation to stress environments. ‘Matador’ TF and Tolerant KBG can maintain acceptable visual quality as long as there is available water in the soil, and they will use up all the water without saving for the future, which makes them suited for planting in deep soil where roots can extend and penetrate to extract more water in the deeper soil profile with a low maintenance program. However, a difference between both entries is that Tolerant ‘KBG’ is able to enter dormancy when water does run out, unlike TF which has no reliable dormancy mechanism. ‘Midnight’ KBG and Susceptible, in contrast, tended to prevent the water loss and preserve water both in soil and plant tissues under drought to wait for the next water resource, which is considered as lower water users.

REFERENCES


CHAPTER 6
DROUGHT TOLERANCE IN WOODY SPECIES SEEDLINGS DIFFERING IN NATIVE WATER AVAILABILITY HABITAT UNDER HIGH DESERT CONDITIONS

ABSTRACT

Three species differing in water availability habitat were tested in responses and mechanisms to cope with drought stress. Two *Acer* species are closely related to sugar maple but different in habitat. *Acer grandidentatum* Nutt. (bigtooth maple; xeric and drought tolerant), *A. macrophyllum* Pursh. (bigleaf maple; mesic and drought intolerant) and *Eucalyptus camaldulensis* Dehn. (red gum; mesic-saline and drought tolerant) seedlings were subjected to two treatments—well-watered and dry down treatments—with a pot-in-pot system under high desert conditions. Irrigation was based on replacement of daily evaporatranspiration loss (ET) using the gravimetric weighing method. For well-watered treatments, water at 120% of ET was applied while 70% - 50% of ET was added for dry down treatment. Measurements were conducted from August to September in both 2010 and 2011. Data collection included ET, stomatal conductance ($g_s$), leaf water potential ($\psi_{leaf}$), photosynthesis, difference between leaf and air temperature ($T_{leaf} - T_{air}$), vapor pressure deficit (VPD), leaf-to-air vapor pressure deficit (LAVPD), osmotic adjustment (OA), elastic adjustment (EA), symplastic water fraction, and relative water content at turgor loss ($\psi_{TLP}$). Plants used several mechanisms to

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1 Coauthored by Nisa Leksungnoen, Roger K. Kjelgren, Richard C. Beeson, Jr., Paul G. Johnson, and Jonathan Carlisle
maintain turgor pressure during a drought period. Mesic species of Eucalyptus and bigleaf maple exhibited similar avoidance mechanisms in response to drought by continuing water uptake from soil based on anisohydric behavior. In addition, Eucalyptus exhibited both OA and EA which contributed to drought tolerant strategies, while bigleaf maple showed only EA. Bigtooth maple, in contrast, exhibited isohydric behavior which tended to conserve water by decreasing water use from ET through stomata control for turgor maintenance along with OA.

INTRODUCTION

Drought is a phenomenon most commonly occurring in arid or semi-arid areas including the Intermountain West, which is considered as high desert conditions (See Chapter 2). Thus, plants have evolved to adapt to low soil water potential, high temperature, and high irradiance with various mechanisms (Bartels and Sunkar, 2005; Chaves et al., 2003; Farooq et al., 2009). However, drought is predicted to extend to much of the world, including moist habitats such as tropical regions (Ainsworth and Ort, 2010; Dai, 2011). Therefore, plants native to wet habitats, which generally are drought sensitive, may have a chance to experience water scarcity in the near future.

Plants adopt many strategies to cope with drought stress in order to maintain normal growth or at least survive during a drought period. An extensive and prolific root system coupled with reducing transpiration via stomatal closure has long been known for a drought avoidance mechanism that helps maintain water uptake (Hinckley et al., 1979; Levitt, 1972). Maintaining turgor pressure of cells is the vital key for surviving under
drought stress. Adjusting osmotic pressure and/or cell elasticity are considered as drought
tolerance mechanisms to maintain turgor pressure (Farooq et al., 2009; Verslues et al.,
2006).

Cell elastic adjustment (EA) can be calculated from the slope of the PV-curve
(Steudle and Zimmermann, 1977; Tyree and Hammel, 1972) which represents the ratio of
the change in cell turgor to that in the relative cell volume (Rada et al., 1989; Saito et al.,
2006). An elastic cell (small $\varepsilon$) will sustain a smaller decrease in turgor potential as a
given volume of water is lost than will a more rigid cell (large $\varepsilon$) (Joly and Zaerr, 1987).
Rigid cells, in contrast, allow a large difference in water potential between soil and leaves
to be produced with relatively little water loss which would, in turn, increase water
uptake (Bolaños and Longstreth, 1984). However, both high and low tissue elasticity
contributes to turgor maintenance during drought stress (Fan et al., 1994; Joly and Zaerr,

Stomatal aperture is positively related to turgor pressure of the guard cells that
have pores through which water moves in or out, due to changes in guard cell water
potential gradient (Buckley, 2005). Thus, when drought occurs, water potential in guard
cells is less negative and leads to water moving out of guard cells, resulting in stomata
pore closure. It has long been known that stomata regulate CO$_2$ uptake and water vapor
loss; thus closing stomata under drought stress can decrease both photosynthesis and
transpiration (Fry and Huang, 2004; Zeiger, 1983).

Isohydric and anisohydric behaviors have been used to describe the control of
stomata via hydraulic signal (Tardieu and Simonneau, 1998). In a typical anisohydric
behavior, both leaf water potential ($\psi_{\text{leaf}}$) and stomatal conductance ($g_s$) decline with decreasing soil water potential (drier soil). In contrast, isohydric species control gas exchange in such a way that daytime leaf water potential does not depend on soil water status (Lambers et al., 1998; Tardieu and Simonneau, 1998). In woody plants, upland drought-tolerant species often show anisohydric behavior which is adapted to fit in water limited environments by developing deep and extensive root systems, osmotic adjustment, and sustaining capacity for photosynthesis and resistance to protoplasmic injury (Pallardy, 2008). Riparian species, generally considered drought sensitive, show isohydric behavior (Loewenstein and Pallardy, 2002).

Trade-offs between anisohydric and isohydric behavior involve gas exchange rate and cavitation. During drought, cavitation occurs when xylem becomes air-filled or embolized due to high suction of roots which bring air to replace water in the xylem (Sperry et al., 2002). As a result, the transpiration water stream is disconnected between roots to shoots. In anisohydric plants, gas exchanges are maintained much further at lower soil water potential (West et al., 2008). However, anisohydric plants will trade-off the normal water use for growth maintenance, with excessive cavitation (Sperry et al., 2002) which causes catastrophic xylem dysfunction (Jones and Sutherland, 1991). In contrast, isohydric plants reduce the gas exchange in order to save water use, conserve water in the plants, and avoid cavitation, resulting in slow growth compared to anisohydric plants. Yet, they can survive under prolonged drought without any injury to the cells. Their growth will resume rapidly after receiving water (West et al., 2008).

Stomatal closure with increased vapor pressure deficit of ambient air (VPD) is
observed in many species to moderate transpiration under high evaporative demand (Bates and Hall, 1981; Monteith, 1995; Turner et al., 1984). When stomata close in response to drought, leaf temperature can increase by several degrees C, thereby increasing leaf-to-air vapor pressure deficit (LAVPD), which is a driving gradient for transpiration. Thus, LAVPD is both a cause and a consequence of water movement through the plant (Tardieu and Simonneau, 1998). Large-leaved species, typically from mesic habitats, could reach rapid stomatal closure from small changes in soil drying and decreased internal water potential, resulting from leaf heating due to a large boundary layer that limits convective heat loss (Kjelgren et al., 2011; Turner, 1981)

Bigleaf maple (Acer macrophyllum Pursh.) is native to mesic habitats and classified as a drought intolerant species (Minore and Zasada, 2012). It would be interesting to understand the responses and mechanisms for coping with drought in plants that are genetically sensitive to water deficit, in order to discover the potential to acclimate to drought, as it now extends all over the world (Dai, 2011). In addition, bigleaf maple is closely related to sugar maple, which is also close to bigtooth maple (A. grandidentatum Nutt.). However, bigtooth maple is native to xeric regions and drought tolerant (Barker, 1977; Emad, 2005). Thus, comparison of closely related species that differ in habitat will minimize inter-species differences and provide a piece of information about the similarities and/or discrepancies about drought responses and mechanisms.

Both drought and salinity stresses lead to cellular dehydration, which causes osmotic stress (Bartels and Sunkar, 2005). Thus, plants may use common pathways in
response to those stresses (Pastori and Foyer, 2002; Tuteja, 2007) As a consequence, salinity tolerant plants are likely to be drought tolerant and vice versa. The objectives of this paper were to understand the responses and mechanisms to drought stress under high desert conditions of closely related maple species (bigleaf and bigtooth maple), compared to a very well-known drought and salinity tolerant species also native to mesic-saline habitats, as a control check (*Eucalyptus camaldulensis* Dehn., red gum Eucalyptus).

**MATERIALS AND METHODS**

This study began with manual irrigation in 2010 (23 August to 29 September). Later in 2011 (29 August to 3 October), lysimeters with load cells were used to provide an automatic irrigation and evapotranspiration (ET) calculation.

**Plant materials**

*Acer grandidentatum* Nutt. (bigtooth maple) and *A. macrophyllum* Pursh. (bigleaf maple) were obtained bareroot from a local nursery in April 2010. Plant roots were soaked overnight to ensure proper hydration before planting in 9.6-L pots (#3, Polytainer, Nursery Supplies, Inc., Orange, CA) filled with Sphagnum Peat Moss medium (Sunshine mix #1, SunGro Horticulture Inc., Bellevue, WA). Plants were fertilized with 78 g of a 12.7N – 7.6P – 10.2K controlled-release fertilizer (Osmocote 15-9-12 last for 3–4 months, Scotts Company Inc., Marysville, OH). All pots were kept in a cold frame (Teaching greenhouse, Logan, Utah) for 3 months (April – June 2010) with daily manual irrigation.
*Eucalyptus camaldulensis* Dehnh. (red gum) seedlings were obtained from seed germination on germination paper (Seedburo Equipment Company, Des Plaines, IL) with tap water under condition at 25°C/15°C day/night temperature with natural light in the greenhouse (Research greenhouse, Logan, Utah) for 2 months (April – May 2010). Then, seedlings were transferred to 4-L pots (True#1, Polytainer, Nursery Supplies, Inc., Orange, CA) filled with the same medium and fertilizer (105 g) as *Acer* species and kept in the greenhouse under the same conditions in seed germination for 2 months (May – June 2010).

In June 2010, 10 uniform plants of each species were selected from the cold frame and greenhouse, and transplanted into 13.75-L pots (#5 Squat, Polytainer, Nursery Supplies, Inc., Orange, CA) filled with the same medium and fertilizer (188 g). Then, plants were moved to the Greenville Research farm (North Logan, Utah) under 50% shade cloth in order to acclimate to the field environments until the experiment started on 23 August 2010. All pots were covered with 2.54 cm-thick Styrofoam to minimize evaporation from soil and prevent as little water as possible from other sources getting into the pot. The pot-in-pot system using 13.75-L pots (#5 Squat, Polytainer, Nursery Supplies, Inc., Orange, CA) as socket pots was applied to prevent high root temperature due to direct sun exposure to pots. The system consisted of two rows with 15 pots in each row. Pots were spaced 0.15 m and 1.52 m within and between rows.

In June 2011, *Acer* species plants were selected from 13.75-L pots from the previous year and transplanted into 34-L pots (EG4004 #10, Grip-Lip, Nursery Supplies, Inc., Orange, CA) for lysimeters. *E. camaldulensis* seeds were germinated and kept in the
same conditions as described in 2010, and then also transplanted into 34-L pots. All pots were transferred to lysimeters at the Greenville Research farm (North Logan, Utah) until the experiment started on August 29. A second pot-in-pot system with 50-L pots (GL6900 #15, Grip-Lip, Nursery Supplies, Inc., Orange, CA) as socket pots was also used. The space between socket and liner pots was filled with wood chips and the surface of pots was also covered with wood chips to minimize ET from medium. This system consisted of three rows with 12 pots in two rows and six pots in one row. Pots were spaced 0.3 m and 1.22 m within and between rows.

**Treatments and irrigation application**

Two treatments were applied in this study: well-watered and dry down treatments. Five plants of each species were randomly assigned to each treatment (30 plants total). The amount of irrigation water was based on weight loss each day from evapotranspiration (ET). For the well-watered treatment, irrigation was applied to 120% of ET to ensure root zone saturation. In 2010, the dry down treatment initially applied 70% of daily ET for Eucalyptus and bigleaf maple, while 60% of ET was applied to bigtooth maple due to the difference in leaf area. After a week, the deficit level was decreased to 50% of ET in Eucalyptus and bigleaf maple, with no additional water applied to bigtooth maple in order to increase the level of drought stress. In 2011, the dry down rate was at 60% of ET for all three species initially. After a week, irrigation was withheld for bigtooth maple and applied at 60% of ET for Eucalyptus and bigleaf maple.

In 2010, plants were weighed and irrigated. Initially all containers were irrigated
to saturation, then allowed to drain for 2 hours and re-weighed to obtain container capacity. Thereafter, containers were weighed at 8:00 am daily to obtain weight loss from the previous day (ET) and the water was added by hand according to ET of each pot, following treatments described above.

In 2011, lysimeters which consisted of 30 load cells (S-type hanging load cell model SSM-AJ-500, Interface Inc., Scottsdale, AZ) were attached to the top of a 2-m tall steel I-beam (Beeson, 2011). The other end of each load cell was attached to a big boa I-beam key lock that connected to a 2 m-long chain. Liner pots with plants were hung by chain about 3 cm above the socket pots allowing liner pots to move freely without touching the socket pots. Load cells were connected to two multiplexers (AM32, Campbell Scientific, Inc., Logan, UT) that were connected to and controlled by a data logger (CR1000, Campbell Scientific, Inc., Logan, UT) set up to obtain an automatic weight loss and calculate ET for each pot daily (Appendix 1). Mass of each pot was recorded every 30 min. All plants were automatically irrigated, after ET was calculated, using Maxijet spraying pot stake of 39.7 liter per hour at 20 psi with 160˚ spray pattern (Maxijet, Inc., Dendee, FL) at midnight.

**Measurements**

Evapotranspiration

Actual evapotranspiration ($ET_A$) of each lysimeter plant was calculated daily by mass difference between at 5:00 am and 11:00 pm MDT. Reference evapotranspiration ($ET_0$) was obtained from an onsite weather station in Greenville Research farm using the
Campbell Scientific, Inc. Daily ET_A (g) data were normalized by leaf area (cm^2) which yielded in units of depth (mm). Leaf area was measured and estimated by regression technique. Twenty-five leaves of each species were measured in length and width (cm) and then fed to a portable leaf area meter (Model Li-3000, LI-COR, Lincoln, NE) to obtain leaf area. The relationship between length times width and leaf area was established by regression to estimate the leaf area of whole plant. Then, length and width of each leaf in all containers were measured and estimated for leaf area at the beginning of the experiment. All species were inquiescence during the experiment. ET_A (mm) was further normalized by dividing by ET_0 (mm) as known by water need index (WNI) (Beeson and Brooks, 2008). In order to compare values of three species, ET_A/ET_0(WNI) was expressed as percent of dry down plants relative to well-watered plants and the data were plotted over time. In addition, a total number of observations (frequency distribution) of relative ET_A/ET_0 in each species were plotted.

**Stomatal conductance**

Stomatal conductance (g_s) measurements were made twice every day—midmorning from 10:00 am to 11:00 am MDT and midday from 2:00 pm to 3:00 pm MDT using a leaf porometer (Model SC-1, Decagon Devices, Inc., Pullman, WA, USA). An intact leaf was inserted with the abaxial side (bottom) of the leaves facing the porometer chamber. By using the instrument’s automatic mode, the g_s was measured in 30 s. Five separate measurements were made from each species-treatment combination.
and averaged for a final value. \( g_s \) was plotted over time in midmorning and midday each year.

Leaf water potential

The lower intact leaf of each species-treatment combination was wrapped with aluminum foil at 1:00 pm MDT every other day. The leaf petiole was cut at 3:00 pm MDT to ensure that leaf was in equilibrium with soil water potential at midday. All 30 samples were immediately stored in an ice bag after cutting to prevent transpiration and rapidly transferred to the lab to measure leaf water potential (\( \psi_{\text{leaf}} \)). Each leaf was unwrapped and the petiole was cut again to ensure a clear cut end, and then it was placed into a pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Nitrogen gas was slowly applied to increase the chamber’s atmospheric pressure until water appeared at the cut end of the petiole. That pressure was used as \( \psi_{\text{leaf}} \). Five separate measurements were made from each species-treatment combination and averaged for a final value. Then the ratio of dry down to well-watered of \( g_s \) versus \( \psi_{\text{leaf}} \) of each species-treatment combination was plotted.

Photosynthesis

Photosynthesis was measured using a portable photosynthesis system (Model LI-6400, LI-COR, Lincoln, NE). There was an instrumental problem in 2010; thus only data in 2011 were presented in this paper. An intact leaf of each species-treatment combination was placed into the fluorescence chamber. The instrument was set with the
flow rate at 500 μmol m\(^{-2}\) s\(^{-1}\), CO\(_2\) at 400 μmol m\(^{-2}\) s\(^{-1}\), and light at 1000-1200 μmol m\(^{-2}\) s\(^{-1}\) with 10\% of blue light. The data were manually logged after photosynthesis, CO\(_2\), H\(_2\)O, and fluorescence were stable. Three replications were measured in weeks 3-5 after the experiment began, under clear sky conditions from 10:00 am to 2:00 pm MDT.

Difference between leaf and air temperature

Ambient air temperature data (T\(_{\text{air}}\)) were continuously collected with a combination of temperature and humidity sensors (model CR500, Campbell Scientific, Logan, Utah, USA) from the onsite weather station at Greenville Research farm. The sensor was scanned every 10 s and averages were recorded every 30 min with a data logger (model CR1000, Campbell Scientific, Logan, Utah, USA). Leaf temperature (T\(_{\text{leaf}}\)) was measured at the same time g\(_s\) was conducted, using a digital thermometer (Model 52-II Dual Input Digital Thermometer, Fluke Corporation, Everett, WA, USA) connected with infrared temperature sensors (Model SI-111, Apogee Instruments, Inc., Logan, UT, USA). The infrared temperature sensor was held 10 cm perpendicular to the leaf. Then, the difference between leaf and air temperature (T\(_{\text{leaf}}\) - T\(_{\text{air}}\)) were calculated.

Vapor pressure deficit and leaf-to-air vapor pressure deficit

Vapor pressure deficit (VPD) and leaf-to-air vapor pressure difference (LAVPD) were calculated as described by Murray (1967) using ambient air temperature, dew point temperature, and leaf temperature from the same onsite weather station in Greenville Research farm. VPD is the difference between saturation vapor pressure and actual vapor
Pressure of ambient air \((e_s - e)\), whereas LAVPD was calculated from the difference between saturation vapor pressure of the leaf using leaf temperature and actual vapor pressure of the ambient air \((e_l - e)\). Then \(g_s\) data were plotted over LAVPD.

Pressure-volume curve

Pressure-volume curve (PV curve) was created as described by Tyree and Hammel (1972) and Hinckley et al. (1980) after all measurements mentioned above were done only in October 2011. All plants were watered and pots were wrapped with plastic, and then kept in a cool room (4°C) with no light for 24 hours in order to resaturate leaves. On the next day, a fully re-saturated leaf was removed from the plant and weighed to obtain the fully turgid weight. Then fresh weights were repeated before and after each pressure chamber reading of water potential were made on the leaf. On each occasion, the chamber was pressurized and depressurized very slowly (less than 0.01 MPa s\(^{-1}\)) using a nitrogen gas supplied pressure chamber (Model 3005HGPL, Soil Moisture Equipment Corp, Santa Barbara, CA). Between readings, the leaf was allowed to transpire freely outside the pressure chamber. After finishing readings, leaves were oven-dried at 80°C for 48 hours and weighed to determine dry weight.

Osmotic potential at full saturation \((\psi_{\text{sat}})\), osmotic potential at turgor loss point \((\psi_{\text{TLP}})\), relative water content at turgor loss point \((\text{RWC}_{\text{TLP}})\), apoplastic water fraction, symplastic water fraction and volumetric elastic modulus \((\square)\) were calculated from the PV curves (Turner, 1981). Osmotic adjustment was calculated as the different between \(\psi_{\text{sat}}\) of treatment plants and the mean \(\psi_{\text{sat}}\) of control plants (Lazarus et al., 2011).
In the pressure chamber, the turgor pressure is reduced to zero by applying the pressure to the leaves. Once the turgor pressure reaches zero, the volume of the water in the cell is related to applied pressure:

\[
\frac{1}{P_c} = \frac{V_s - V}{RTN}
\]

where \( P_c \) is the pressure in the chamber, \( V_s \) is the volume of symplastic water in the turgid leaf, \( V \) is the volume of the symplastic water expressed, \( R \) is the gas constant, \( T \) is the Kelvin temperature and \( N \) is the moles of solute in the leaf. Thus a plot of \( 1/P_c \) against \( V \) should become linear when the turgor pressure becomes zero (PV curve).

Extrapolation of the straight line \( V=0 \), gives the \( \psi_{\text{sat}} \), and the \( \psi_{\text{TLP}} \) is the point at which the water potential and osmotic potential are equal. Extrapolation of the straight line to \( 1/P_c = 0 \), i.e. infinite pressure, gives the total symplastic water in the leaf (\( V_s \)).

Total volume of water in the leaf (\( V_t \)) is determined from the difference between initial turgid weight (TW) and oven-dried weight (DW). Then, the apoplastic water, i.e. water in the cell walls is \( V_t - V_s \). It should also be apparent that the relative water content (RWC) is given by:

\[
RWC = \frac{V_t - V}{V_t} \times 100
\]

and the relative symplastic water content (RSWC) is given by:

\[
RSWC = \frac{V_s - V}{V_s} \times 100.
\]

Further, the volumetric modulus of elasticity (\( \varepsilon \)) is given by:

\[
\varepsilon = \frac{\Delta P}{\Delta RSWC} \times 100
\]
Statistical analysis

The experiment was a completely randomized design with two factors: irrigation regimes (two levels) and plant species (three levels) with five replications, except with three replications for photosynthesis measurement. Treatment effects, species differences, and treatments x species interactions were determined by analysis of variance (ANOVA) according to the GLM procedure by SAS (version 9.0; SAS Institute, Cary, NC). Mean differences were tested with the least significant difference test at a probability level of 0.05. Linear regression lines of the ratio of dry down to well-watered of $g_s$ versus $\psi_{leaf}$ and $g_s$ versus LAVPD were obtained from Sigmaplot program (version 11.0; Systat Software, Inc., San Jose, CA). Slopes of the regression lines were compared using the sum of square calculation from linear and non-linear fitting lines by SigmaPlot with the Snedecor’s $F$ statistic at a probability level of 0.05.

RESULTS

Drought stress apparently occurred in Eucalyptus and bigleaf maple but only slightly in bigtooth maple due to a slower growth rate and smaller leaf area compared to the species. By the end of either experiment, dried-down bigtooth maple weighed 20% less than at saturation weight, while the decrease was 60% for both the Eucalyptus and bigtooth maple (data not shown). Even though irrigation was withheld 22 days to force bigtooth maple to approach severe drought stress, the permanent wilting point was not obtained even after 7 – 8.5 cm $ET_0$ (data not shown). This reduction in growth rate is one of the mechanisms that genetically adapt bigtooth maple to stress and surviving during
unfavorable conditions.

Eucalyptus and bigleaf maple are considered as fast growing species compared to bigtooth maple in that they require abundant water to support elongation mechanisms. The ratio of ET_A/ET_0 expressed as dry down plants relative to well-watered plants exhibited that both Eucalyptus and bigleaf maple initially transpired at a higher rate than ET_0 (relative ET_A/ET_0 > 100%) (Fig. 6-1a-b). Later, when the level of drought stress increased until the relative ET_A/ET_0 reached about 90 to 80, the water use suddenly decreased. The ET of dry down plants was statistically different from well-watered plants when the relative ET_A/ET_0 was 40%, which was considered as a threshold of both Eucalyptus and bigleaf maple (Fig. 6-1a-b, with arrows). This suggests that both species tended to continue using water even the drought stress increased until there was not enough water to support an anisohydric preference, then the water use reduced. Bigtooth maple water use, in contrast, initially decreased right after the dry down treatment began in both years, but later tended to maintain the water use at about 60% (Fig. 6-1a-b).

The distribution of water use was similar in Eucalyptus and bigleaf in that their water use was scattered across all relative ET_A/ET_0 ranges but had the highest peak at relative ET_A/ET_0 higher than 80% (Fig. 6-1c-d). Bigtooth maple had no distribution at relative ET_A/ET_0 lower than 60% while the peak was at about 60 – 80% of relative ET_A/ET_0. Based on the peak of distribution, the results clearly exhibited that both Eucalyptus and bigleaf maple used water in a greater amount than bigtooth maple.
**Fig. 6-1.** Ratio of actual evapotranspiration to reference evapotranspiration expressed as percent of dry down relative to control of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) in (a) 2010 and (b) 2011. The arrows show the date when the relative ET<sub>A</sub>/ET<sub>0</sub> of well-watered plants was statistically different from that of dry down plants at $P < 0.05$.

Number of observations (frequency distribution) of relative actual evapotranspiration over reference evapotranspiration of 3 species in (c) 2010 and (d) 2011.
Stomatal conductance ($g_s$) of dried-down Eucalyptus and bigleaf maple followed the similar trend as ET, in which they maintained open stomata until reaching a threshold and then immediately declined as drought level increased. In contrast, dried-down bigtooth maple was able to maintain $g_s$ at the same level as well-watered plants for an entire experimental period (Fig. 6-2). The dried-down Eucalyptus and bigleaf maple were significantly lower than well-watered when the relative $g_s$ reached 40%, except for midday 2010, which took 20% further to exhibit the difference (Fig. 6.2, with arrows).

Leaf water potential ($\psi_{leaf}$) decreased (more negative) as drought level increased in Eucalyptus and bigleaf maple, while there was no change in bigtooth maple (Fig. 6.3). Dried-down Eucalyptus exhibited significantly lower in $\psi_{leaf}$ than well-watered when its $\psi_{leaf}$ dropped down to about -1.7 to -2 MPa (Fig. 6-3a-b). In the case of bigleaf maple, the threshold where dry down plants exhibited the difference to well-watered plants varied between the two years, which was at 1.2 in 2010 but 2.4 in 2011 (Fig. 6-3c-d).

Both $g_s$ and $\psi_{leaf}$ decreased in response to increasing drought stress. Fig. 6-4 clearly exhibits that Eucalyptus and bigleaf maple $g_s$ continued to decrease, while $\psi_{leaf}$ was maintained until a threshold was reached at about 40% of $g_s$ and 50% of $\psi_{leaf}$ of dry down relative to well-watered plants. After this threshold, stomata almost completely closed (low $g_s$ ratio) while $\psi_{leaf}$ rapidly dropped (high $\psi_{leaf}$ ratio). Those two species could be classified as having anisohydric behavior, as they kept lowering their $\psi_{leaf}$ to extract more water from soil until there was no accessible water remaining, and then dying tissues occurred beginning at leaf margins through to the entire plant. Bigtooth maple, in contrast, showed isohydric behavior in that stomata strongly controlled $\psi_{leaf}$. 
Fig. 6-2. Stomatal conductance expressed as percent of dry down relative to control of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) in 2010 (a) Midmorning and (b) Midday and in 2011 (c) Midmorning and (d) Midday. The arrows indicate when the stomatal conductance of well-watered plants was statistically different from that of dry down at $P < 0.05$. 
Fig. 6-3. Midday leaf water potential of *Eucalyptus camaldulensis* Dehnh. (red gum) in (a) 2010 and (b) 2011, *Acer macrophyllum* Pursh. (bigleaf maple) in (c) 2010 and (d) 2011, and *A. grandidentatum* Nutt. (bigtooth maple) in (e) 2010 and (f) 2011. The arrows exhibit the date when the leaf water potential of well-watered plants was statistically different from that of dry down at $P < 0.05$. 
Fig. 6-4. The relationship between ratio of dry down to control plants in stomatal conductance and leaf water potential of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple). Data points were fitted with linear regression. The same letters in each line are not significantly different at $P < 0.05$.

Photosynthesis decreased as plants experienced drought, but only in Eucalyptus and bigleaf maple, while dried-down bigtooth maple exhibited no change in photosynthesis (Fig. 6-5). The difference in photosynthesis between dry down and well-watered plants occurred at the same time as when ET showed the difference.
Fig. 6-5. Photosynthesis of dry down relative to control plants in 2011 of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple).

Leaf temperature ($T_{leaf}$) of dry down plants of all species was similar to well-watered until significant differences in ET occurred between treatments. Thereafter, $T_{leaf}$ of dry down plants were significantly higher than those of well-watered plant $T_{leaf}$ (data not shown). $T_{leaf}$ in the dry down plants was similar in 2010 but distinguishable in 2011 (Fig. 6-6). In 2011, dried-down bigleaf maple exhibited the highest leaf temperature both in midmorning and midday. Eucalyptus and bigtooth maple $T_{leaf}$ were similar in midmorning (Fig. 6-6c) but it was higher in bigtooth maple than in Eucalyptus in midday (Fig. 6-6d) until severe drought occurred in Eucalyptus when its $T_{leaf}$ increased to the same level as bigleaf maple (Fig. 6-6).
Fig. 6-6. Leaf temperature of dried-down *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) in 2010 (a) Midmorning and (b) Midday and in 2011 (c) Midmorning and (d) Midday.

Well-watered Eucalyptus exhibited the lowest difference between leaf and air temperature ($T_{\text{leaf}} - T_{\text{air}}$) in both years and tended to keep leaf temperature lower than air temperature at midday (Fig. 6-7). As water deficit increased, $T_{\text{leaf}} - T_{\text{air}}$ increased in dry down plants while it tended to decrease in well-water plants. Dried-down bigleaf maple
exhibited the highest $T_{\text{leaf}} - T_{\text{air}}$, followed by dried-down Eucalyptus. In contrast, dried-down bigtooth maple tended to maintain or decrease $T_{\text{leaf}} - T_{\text{air}}$ when drought increased (Fig. 6-7)

**Fig. 6-7.** The difference between leaf and air temperature during dry down period of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) in 2010 (a) midmorning and (b) midday, and in 2011 (c) midmorning and (d) midday. Data points were fitted with linear regression.
$T_{\text{leaf}} - T_{\text{air}}$ decreased toward zero as VPD increased (drier air condition) However, there were no differences among species and also between treatments in the slope of linear regression lines (Fig. 6-8). This suggests that as air became drier, plants tended to increase or at least maintain transpiration rate in order to cool the leaf and keep $T_{\text{leaf}}$ close to $T_{\text{air}}$ in both well-watered and dry down plants and due to leaf sizes among species and/or differences in cuticular conductance.

**Fig. 6-8.** The difference between leaf and air temperature against vapor pressure deficit of *Eucalyptus camaldulensis* Dehn. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) of pooled data over 2 years and both in midmorning and midday. Data points were fitted with linear regression.
Leaf-to-air vapor pressure deficit (LAVPD) was similar between treatments (Fig. 6-9). Increasing $T_{\text{leaf}}$ causes high LAVPD which is the driving gradient for transpiration; thus as LAVPD increases the demand for transpiration would also increase in order to reduce $T_{\text{leaf}}$. Well-watered plants tended to maintain $g_s$ over LAVPD range at about 600 mmol m$^{-2}$ s$^{-1}$ in Eucalyptus and bigleaf maple, and at about 400 mmol m$^{-2}$ s$^{-1}$ for bigtooth maple. As LAVPD increased, dry down $g_s$ decreased in Eucalyptus and bigleaf maple but not in bigtooth maple (Fig. 6-9b). This suggests that $g_s$ in bigtooth maple was not regulated with by drought stress, as apparently occurred in Eucalyptus and bigleaf maple.

Fig. 6-9. The relationship between stomatal conductance and leaf-to-air vapor pressure deficit of *Eucalyptus camaldulensis* Dehnh. (red gum), *Acer macrophyllum* Pursh. (bigleaf maple), and *A. grandidentatum* Nutt. (bigtooth maple) in (a) well-watered plants and (b) dry down plants. Data points were fitted with linear regression. The same letters in each line are not significantly different at $P < 0.05$. 
The PV curve analysis indicated that that bigleaf maple exhibited no difference in water potential at full turgor ($\psi_{sat}$) and at turgor loss point ($\psi_{TLP}$) (Table 6-1). Dried-down Eucalyptus and bigtooth maple exhibited substantially lower (more negative) $\psi_{sat}$ and $\psi_{TLP}$ where turgor loss occurred at -2 MPa in Eucalyptus and -1.2 MPa in bigtooth maple. The turgor loss point in dried-down bigleaf maple was -1 MPa (Table 6-1).

High total symplastic water in the dried-down plants showed the defense mechanism under drought stress. Symplastic water contributes to maintain cell turgor which helps delay turgor loss. However, only Eucalyptus exhibited higher ($P < 0.05$) symplastic water content in dried-down plants compared to well-watered plants. Symplastic water was not different between treatments ($P < 0.05$) in the *Acer* species (Table 6-1).

Relative water content in leaf cells at plasmolysis ($RWC_{TLC}$) indicates the water content when the turgor loss point occurred. Thus, dried-down plants of all species postponed the turgor loss point by reaching plasmolysis at lower water content than well-watered plants. Among species, Eucalyptus and bigleaf maple exhibited lower $RWC_{TLC}$ than bigtooth maple, suggesting that they could maintain turgor pressure at lower water content than bigtooth maple.

Osmotic adjustment (OA) helps maintain turgor pressure by decreasing cell osmotic potential. Dried-down Eucalyptus and bigtooth maple exhibited the ability to osmotically adjust their leaf water potential but in different magnitudes, of which OA in Eucalyptus was higher than in Bigtooth maple (Table 6-1). Bigleaf maple showed no adjustment in this study.
Changes in cell wall elasticity also contribute to turgor maintenance. Elastic cell wall, expressed as low modulus elasticity (low $\varepsilon$), occurred in dry down plants indicating that they maintained more water at full turgor, hence, their volume could decrease more before the turgor-loss point was reached. However, $\varepsilon$ in bigtooth maple showed no significant difference between treatments and had among the highest $\varepsilon$. At these levels of $\varepsilon$, cells of bigtooth maples relied on rigid cell walls to generate low $\psi_{\text{leaf}}$ for greater scavaging of soil water.

**DISCUSSION**

Inter-and intra-specific variation in mechanisms to cope with drought has been encountered in tree species (Gindaba et al., 2004; Lemcoff et al., 2002; Merchant et al., 2007). Stomata control over transpiration rate and leaf water potential and extended root system to extract water from deeper soil layer contributed to drought avoidance and tolerance mechanisms (Bsoul et al., 2006; Lemcoff et al., 1994; Lemcoff et al., 2002; Taneda and Sperry, 2008).

Our results exhibited that Eucalyptus and bigleaf maple postponed dehydration during drought periods by maintaining water uptake, which is considered a drought avoidance mechanism (Farooq et al., 2009; Lemcoff et al., 2002). However, when drought stress increased and reached threshold point (at 40% relative $\text{ET}_A/\text{ET}_0$) (Fig. 6-1), both species rapidly experienced tissue desiccation starting from leaf margin, to the whole leaf, and then dieback of entire plants. Bigtooth maple, in contrast, classified as a drought tolerant species, controlling water use by closing stomata and reducing
Table 6-1  The pressure-volume curve analysis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Eucalyptus</th>
<th>Bileaf maple</th>
<th>Bigtooth maple</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well-watered</td>
<td>Dried-down</td>
<td>Well-watered</td>
</tr>
<tr>
<td>%Apoplastic$^z$</td>
<td>76.73ab$^s$</td>
<td>54.85c</td>
<td>82.49ab</td>
</tr>
<tr>
<td>%Symplastic$^y$</td>
<td>23.26bc</td>
<td>45.16a</td>
<td>17.5bc</td>
</tr>
<tr>
<td>$\psi_{sat}$ (MPa)$^x$</td>
<td>0.74bc</td>
<td>1.16a</td>
<td>0.54c</td>
</tr>
<tr>
<td>$\psi_{TLP}$ (MPa)$^w$</td>
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<td>2.02a</td>
<td>0.86c</td>
</tr>
<tr>
<td>RWC$_{TLP}$ $^v$</td>
<td>0.94b</td>
<td>0.91c</td>
<td>0.95b</td>
</tr>
<tr>
<td>$\sigma$ (MPa)$^u$</td>
<td>1.31ab</td>
<td>0.46c</td>
<td>1.07b</td>
</tr>
<tr>
<td>OA (MPa)$^l$</td>
<td>0.42a</td>
<td>0.00c</td>
<td>0.24b</td>
</tr>
</tbody>
</table>

$^z$Apoplastic water fraction

$^y$Symplastic water fraction

$^x$Osmotic potential at full turgor (MPa)

$^w$Osmotic potential at plasmolysis (loss turgor point) (MPa)

$^v$Relative water content at plasmolysis (loss turgor point) (m$^3$ m$^{-3}$)

$^u$Modulus elasticity (MPa)

$^l$Osmotic adjustment which calculated from the difference of osmotic potential at full turgor between well-watered and dried-down plants (Lazarus et al., 2011).

$^s$Values in each row followed by the same letter are not significantly different at the $P < 0.05$. 
transpiration; this mechanism is important in maintaining turgor pressure over the drought period (White et al., 2000).

The water use distribution (Fig. 6-1c-d) exhibited that Eucalyptus and bigleaf maple transpiration was greater than bigtooth maple, resulting in consistency of maintaining leaf temperature close to the air (Fig. 6-7). Even though the leaf size of species were different (the smallest in bigtooth and the biggest in bigleaf maple), the $T_{\text{leaf}} - T_{\text{air}}$ was close among species-treatment combinations. Generally, large leaf size has higher leaf temperature compared to a smaller leaf, due to a large boundary layer resulting in slow heat dissipation (Gate, 1968) which evidently occurred in bigleaf maple in this study. However, in our study, large-leafed Eucalyptus maintained leaf temperatures close to the air through a large transpiration rate (Fig. 6-1) and arranged leaf angle in the range 60-80˚ from the horizontal with turning the leaves with their edges towards the sun during drought periods, indicating the adaptation to reduce sensible head load at high irradiance (Whitehead and Beadle, 2004). The smaller leaf size of bigtooth maple kept its leaf temperature close to air temperature by rapid heat exchange with the environment due to a low boundary layer (Gate, 1968).

Both Eucalyptus and bigleaf maple are native to mesic habitats, which resulted in similar avoidance mechanisms as compared to bigtooth maple, which is from a more xeric habitat. Their responses to drought could be classified as anisohydric behavior (Fig. 6-4) as similar to a tropical rain forest species reported by Kjelgren et al. (2009) which can survive brief dry periods but is unsuited to low water landscapes under minimal or no irrigation. The apparent use-it-or-lose-it water use behavior of both species could rapidly
deplete root zone water until no water is available, leading to dead tissues. This suggests that both species may not survive in long drought period regions, such as in the Intermountain West climate, due to their aggressive water use behavior. Anisohydric Eucalyptus and bigleaf maple exhibited correlation with losing turgor at lower RWC (Table 6-1) compared to isohydric bigtooth maple (0.91 vs. 0.95 cm$^3$ cm$^{-3}$), which allowed them to grow further before cells lost turgor pressure.

Bigtooth maple applied isohydric behavior under drought stress, thus it was classified as drought tolerant due to the mechanism of controlling water use and maintaining water in the cells (Bsoul et al., 2006). Its stomata had strong control over $\psi_{\text{leaf}}$ which was indicated by maintaining $\psi_{\text{leaf}}$ near 100% (ratio of $\psi_{\text{leaf}}$ at 1) while $g_s$ declined (Fig. 6-4). The conservative water use behaviors in bigtooth maple would make it survive in xeric regions with low rainfall and long periods of drought. In addition, it would be suitable for low-water landscapes with low irrigation (Kjelgren et al., 2009).

Loewenstein and Pallardy (1998, 2002) suggest that mesic trees are more inclined towards isohydric behavior while xeric plants are more anisohydric. Our results exhibit the contradiction that Eucalyptus and bigleaf maple (mesic habitat) showed anisohydric behavior while bigtooth maple (relative xeric habitat) exhibited isohydric behavior (Fig. 6-4). Bigtooth maple has been reported to be anisohydric in natural populations adjacent to the roadside in Salt Lake, Utah over dry seasons (Taneda and Sperry, 2008). A sugar maple population from a xeric area also showed anisohydric behavior with increasing xylem sap abscisic acid (ABA) in response to drought (Loewenstein and Pallardy, 1998). This suggests that the inter-and intra-specific variation among and within species appear
to play an important role on identifying the behavior (Schultz, 2003; Sperry and Saliendra, 1994).

However, the Bsoul et al. (2006) study concluded that under limited root penetration bigtooth maple tended to express anisohydric behavior, which agreed with our results as the experiment was conducted under confined container conditions. Thus, the response of bigtooth species to drought may vary, dependent upon the ability of roots to extract water. If a plant’s roots are able to easily access water (i.e. in the field where roots can penetrate to deeper soil layer), anisohydric behavior could be evolved in those populations. In contrast, under limited root expansion (i.e. in a planting container) isohydric behavior would be preferred in order to save water.

Eucalyptus and bigleaf maple also showed stomata sensitivity to dry air under drought stress (Fig. 6-9) by closing their stomata in response to increasing leaf-to-air vapor deficit (LAVPD), thus avoiding desiccation (Monteith, 1995; Maroco et al., 1997). In contrast, bigtooth maple seemed to be insensitive to LAVPD, indicated by maintaining a g_s over LAVPD range closed to well-watered plants (Fig. 6-9). The weak response to LAVPD in bigtooth maple could be due to an isohydric behavior in which less water was spent, resulting in high water content remaining in the medium, same as in well-watered (Maroco et al., 1997).

Even though both Eucalyptus and bigleaf maple responded similarly under drought stress, the mechanisms to cope with drought may be somewhat different between two species because the Eucalyptus habitat is also saline (Boland et al., 2006). Due to the cross-tolerance between drought and salinity stresses, Eucalyptus may inheritably possess
more varieties of stress tolerant mechanisms compared to bigleaf maple (Pastori and Foyer, 2002; Tuteja, 2007).

Plants can adapt to drought by increasing osmotic adjustment (OA) and/or elastic adjustment (EA). Our results indicated that Eucalyptus possessed both mechanisms while bigtooth maple showed only OA, and bigleaf maple exhibited only EA during the drought period (Table 6-1). Due to the lack of OA in bigleaf maple, tissue desiccation rapidly occurred compared to Eucalyptus, which showed a two times lower turgor loss point (-2 MPa vs. -1 MPa) (Table 6-1). Thus the lower \( \psi_{\text{leaf}} \) in bigleaf maple over a drought period (Fig. 6-3) would only indicate the leaf tissue desiccation and not imply OA. Our results suggest that Eucalyptus could maintain cell turgor longer than bigleaf maple under the same drought stress level, due to OA. As a consequence, bigleaf maple cells would collapse due to turgor pressure loss before Eucalyptus did at the same soil water potential.

Elastic adjustment (EA) (more elastic cell wall) has been identified as an important mechanism for drought tolerance in tree species (Joly and Zaerr, 1987; Lenz et al., 2006; White et al., 2000). All species in this study showed EA under drought stress, but less pronouncedly in bigtooth maple (Table 6-1). There is no documentation of EA in bigtooth maple and bigleaf maple. However, sugar maple, a closely related species, has also been reported to have a more rigid cell wall under drought stress (Ellsworth and Reich, 1992; Tyree et al., 1978) as well as red maple (Nash and Graves, 1993). Under the salinity stress of our study (Chapter 3), bigtooth maple experienced severe salt concentration indicated by leaf burn symptoms. It indicated more elastic cell walls
compared to the control treatment, which was similar to this study in both bigtooth and bigleaf maple.

Eucalyptus has been reported to decrease in elastic cell walls (more rigid) under drought stress both in onsite natural habitat (White et al, 2000) and in greenhouse experiments (Lemcoff et al., 2002; Merchant et al., 2007). This could explain the complexity in Eucalyptus for coping with stresses. It may use many strategies to survive different stress levels or types of stress. In our salinity stress experiment (Chapter 3), the Eucalyptus cell wall was more rigid under high salinity concentration (12 dS m$^{-1}$) which was considered as mild or no stress for Eucalyptus. In this study, severe drought stress occurred causing wilting and dying leaves; almost all plant symptoms could be contributed to different strategies in EA than in other studies. Because the PV curve was conducted at the end of the experiment when plants had already passed the permanent wilting point, we may have missed the response of the cell wall before this fatal point.

However, both high and low tissue elasticity could add to turgor maintenance during drought stress (Fan et al., 1994; Joly and Zaerr, 1987; Zimmermann, 1978). A rigid cell wall will decrease in turgor pressure per unit of water loss more than an elastic cell wall; thus, its water potential is lower. As a consequence, soil-leaf water potential gradients increased and thereby promoted water uptake from drying soil in order to maintain turgor pressure (Bowman and Roberts, 1985). An elastic cell wall provides the cells with a high resistance to short-term fluctuations and will sustain a smaller decrease in turgor pressure as a given volume of water loss, which contributes to turgor maintenance (Joly and Zaerr, 1987; Zimmermann, 1978).
Overall, under limited root expansion and high desert conditions, Eucalyptus tolerance mechanisms consist of continuing water uptake from soil based on anisohydric behavior, decreasing leaf water potential via OA, and maintaining turgor via EA. Like Eucalyptus, bigleaf maple also has the anisohydric behavior of extracting water from soil but it has no ability for OA; thus it maintains turgor with EA. In contrast to both former species, bigtooth maple reduces water use through stomata control in order to preserve water for turgor maintenance along with OA.

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Emad, Y.B. 2005. Salinity responses of bigtooth maples native to arid environments. A dissertation of New Mexico State University, Las Cruces, NM.


CHAPTER 7

CONCLUSION

Our results demonstrate that drought tolerance may not necessarily be related to salinity tolerance, especially in turfgrasses. In turfgrasses, salinity tolerant ability was identified based on dosage required to kill 50% of plants (Robins et al., 2009). Then, the salinity experiment was conducted to confirm the previous study. The results showed that Susceptible KBG was the least salinity tolerant, as expected, due to high ion uptake and transport to the shoot with no lowering of leaf water potential ($\psi_{\text{leaf}}$) (Figs. 3-9 and 3-10). However, ‘Midnight’ KBG, which was classified as having moderate salinity tolerance, had an equal tolerance to that of Tolerant KBG and ‘Matador’ TF, which were classified as salinity tolerant. They exhibited the high ratio of K$^+$.Na$^+$ and could lower their $\psi_{\text{leaf}}$, which could be linked to osmotic adjustment mechanisms.

We hypothesized that salinity tolerance could be the same as drought tolerance based on the similar osmotic stress caused by salinity and drought. The results exhibited that Susceptible KBG was as drought tolerant as other entries based on visual appearance (Fig. 5-2). High salt concentration uptake could be the factor that caused this entry to be sensitive to salinity while tolerating drought. Thus, the assumption of cross-tolerance between drought and salt may not be valid at high salt concentrations in turfgrasses.

As the conditions changed from confined root containers in the greenhouse to deep-soil field conditions, TF maintained acceptable visual appearance though a deep root system that extracted more water from deeper soil while KBG went dormant when
stomatal conductance dropped at about 50% of well-watered plants (Fig. 2-4). This indicated different strategies to cope with drought between species under different conditions.

In the case of woody plants, species were selected based on water available habitats which infer drought tolerant ability. Bigleaf maple is a mesic plant and closely related to bigtooth maple, which is native to xeric habitats, while red gum (Eucalyptus) is native to mesic and saline habitats. Again, in order to test the cross-tolerant hypothesis, all species were studied under both drought and salinity stresses. The results were slightly different from those of turfgrass. Bigtooth maple, expected to be drought tolerant, showed both drought and moderate salinity tolerance, while bigleaf maple was sensitive to both drought and salinity. Eucalyptus exhibited salinity tolerance but moderate drought tolerance based on visual appearance.

Overall, common mechanisms that contributed to both salinity and drought stress tolerance in the turfgrasses and woody species in this study were deep root systems (dehydration avoidance) and osmotic adjustment (dehydration tolerance) (Pallardy, 2008). The dehydration avoidant plants seemed to maintain normal metabolisms and continue growing under stresses while dehydration tolerant plants spent most of their energy lowering osmotic potential, resulting in surviving rather than growing normally (Munns, 1988). The responses and mechanisms of turfgrasses and woody species under both salinity and drought stresses were presented in Table 7-1.
Table 7-1. Summary of responses and mechanisms in turfgrasses and woody species under both salinity and drought stresses.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stresses</th>
<th>Acceptable visual appearance</th>
<th>Stomatal behaviors</th>
<th>Salt exclusion</th>
<th>OA(^z)</th>
<th>EA(^y)</th>
<th>Water use</th>
<th>Remarks</th>
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<tr>
<td></td>
<td>Drought</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>Consume</td>
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<td>Anisohydric</td>
<td></td>
<td>No</td>
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<td>Yes</td>
<td>Elastic</td>
<td>Conserve</td>
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\(^z\) Osmotic adjustment from PV-curve analysis

\(^y\) Elastic adjustment from PV-curve analysis
REFERENCES


APPENDIX
Appendix 1 Program for lysimeters with load cells (CHAPTER 6)

'CR1000 Series Datalogger
'Original program author: RC Beeson

'C1 ........ Relay driver
'C2 ........ Relay driver
'C3 ........ Relay driver
'C4 .......... mux1 reset
'C5 .......... mux1 clock
'C6 .......... mux2 reset
'C7 .......... mux2 clock
'C8
'VX1 ....... mux1 com1
'VX2 ....... mux2 com1
'VX3

'Flag definitions for lysimeters:

'Flag 1: Program on/off bypass switch. When low, suspends program processing
'Flag 2: Instant calculation of lys mass with first pass of program. Used in irrigation shutdown routine and for new calibration of load cell.
'Flag 3: Indicates end of 5 count Loop For lys mass AND trigger output Table meanmass.
'Flag 4: Signal flag. Irrigation shutdown subroutine exceeded 15 min time limit.
'Flag 6: not used
'Flag 7: Initiates midnight calculation of ETA and night irrigation volume setup.
'Flag 8: Switch to turn on (F_8=true) or off (F_8=false) midday irrigation.
'Flag 9: Signal flag. Successful progression through midnight ETA cal and irr setup.
'Flag 10: Signal flag. Successful progression through midday irrigation setup.
'Flag 11. Signal flag. Irrigation startup subroutine progressed. (Irrneeded > 0.2 kg).

'variable labels

'lys_mass = lysimeter weight in Kg
'mass_500 = lysimeter weight at 5:00 am
'mass_2200 = lysimeter weight at 10:00 pm
'cd16cntl = solinoid valve state value stored in source table for sdmcd16 relay driver control
'mass = raw lysimeter reading in mV
'total_ETa = PETa2400 + post1pm_massinc
'post1pm_massinc = post 1:00 pm increase in weight (weight increase from midday watering)
'totalirr = total irrigation
'ETa_1pm = ETa
'preirr_mass = pre irrigation weight (weight at midnight)
'cumirrmass = cumulated irrigation weight (used to shut down irrigation)
'parirrvol = total daily irrigation water divided by 3 (irrigation applied in 3 waterings)
'door_open = NEMA door status (used to determine when wiring changes or manual data dumps/program changes are made)
'door_output = used in output of door_open variable

Const off = 0
Const on = -1
Const run = 1

Public batt_volt, ptemp_c, lys_mass(32), mass_500(32), mass_2300(32), CD16cntl(32), mass(32)
Public Total_ETa(32), Post1pm_massinc(32), Totalirr(32), ETA_1pm(32),
preirr_mass(32), cumirrmass(32)
Public Partirrvol(32), middaydelay As Long, irrneeded(2), irr_on(2)
Public switch(7) As Boolean, Flag(12) As Boolean,
Public lcmult(32), lcoffset(32)

'Declare Other Variables
Dim PEta2400(32), lsysloopcount, i As Long
Dim cd16status(2), lcnum(32),

'Act as logic switches
Alias switch(1) = readlysimeters
Alias switch(2) = irrinindicator
Alias switch(3) = shutdownswitch
Alias switch(4) = startupswitch
Alias switch(5) = midday_signal
Alias switch(6) = endirrcyc
Alias switch(7) = irrbypass

'Define Data Tables

'Stores lysimeter masses every half hr
DataTable (halfstor, true,-1)
    DataInterval (0,30,Min,-1)
    Sample (32, lys_mass(), IEEE4)
EndTable
'Calculates mean load cell millivolts (used as intermediate data storage when flag 2 or 3 is high)
DataTable (meanmass,flag(3),3)
    Average (32, mass(),IEEE4,0)
EndTable

'Stores daily values
DataTable (dailysum,flag(7),400)
    Sample (32,Total_ETa(),IEEE4)
    Sample (32, mass_500(),IEEE4)
    Sample (32, Post1pm_massinc(),IEEE4)
EndTable

'Irrigation shutdown subroutine
Sub irrshutdown
    For i = 1 To 32 Step 1
        cumirrmass(i) = lys_mass(i) - preirr_mass(i)
        If cumirrmass(i) < 0 Then
            cumirrmass(i) = 0
        EndIf
    Next i
    For i = 1 To 32 Step 1
        If cd16cntl(i) = run AND partirrvol(i) >= cumirrmass(i) Then
            cd16cntl(i) = run
        Else
            cd16cntl(i) = off
        EndIf
    Next i
    MaxSpa (irr_on,32,cd16cntl(1))
    'Override setting to prevent irrigation events longer than 15 min.
    'Should be changed if "Overirrigate" is on and irrigation is working well
    If TimeIntoInterval (15,30,Min) Then
        If irr_on(1) = run Then
            flag(4) = true
            If shutdownswitch = on Then
                For i = 1 To 32 Step 1
                    cd16cntl(i) = off
                Next i
            EndIf
        EndIf
        flag(12) = true
    EndIf
EndIf
EndIf
EndIf
flag(12) = true
' Resets variables for next irrigation
If irr_on = 0 Then
shutdownswitch = off
startupswitch = false
irrindicator = off
irrbypass = off
If midday_signal = on Then	enirrcyc = on
EndIf
EndIf
EndSub

'Irrigation startup subroutine
Sub irr_start
For i = 1 To 32 Step 1
    If partirrvol(i) >= 0.2 Then
cd16cntl(i) = run
    Else
        cd16cntl(i) = off
    EndIf
Next i
startupswitch=off
irrindicator = on
irrneeded = off
irrbypass = on
flag(2) = true
EndSub

'Main Program
BeginProg

'Calibration constants for load cells. Where mult = slope and offset is constant value
'NOTE: calibration must be in kg, not grams!!!
lcmult(1)=35.791 : lcoffset(1)=-0.0100
lcmult(2)=35.844 : lcoffset(2)=0.153
lcmult(3)=35.709 : lcoffset(3)=-0.372
lcmult(4)=35.939 : lcoffset(4)=0.00274
lcmult(5)=36.193 : lcoffset(5)=0.265
lcmult(6)=35.860 : lcoffset(6)=-0.474
lcmult(7)=35.936 : lcoffset(7)=0.0310
lcmult(8)=36.369 : lcoffset(8)=0.240
lcmult(9)=36.113 : lcoffset(9)=0.249
lcmult(10)=35.800 : lcoffset(10)=0.0118
lcmult(11)=36.088 : lcoffset(11)=0.0130
lcmult(12)=36.085 : lcoffset(12)=-0.527
lcmult(13)=35.777 : lcoffset(13)=-0.487
lcmult(14)=35.997 : lcoffset(14)=-0.438
lcmult(15)=35.904 : lcoffset(15)=0.223
lcmult(16)=35.844 : lcoffset(16)=-0.369
lcmult(17)=36.112 : lcoffset(17)=0.241
lcmult(18)=35.981 : lcoffset(18)=-0.487
lcmult(19)=36.027 : lcoffset(19)=-0.287
lcmult(20)=35.944 : lcoffset(20)=-0.195
lcmult(21)=35.623 : lcoffset(21)=0.241
lcmult(22)=35.816 : lcoffset(22)=0.107
lcmult(23)=36.014 : lcoffset(23)=-0.406
lcmult(24)=35.750 : lcoffset(24)=-0.354
lcmult(25)=36.054 : lcoffset(25)=0.318
lcmult(26)=36.040 : lcoffset(26)=-0.359
lcmult(27)=35.840 : lcoffset(27)=-0.489
lcmult(28)=35.899 : lcoffset(28)=-0.177
lcmult(29)=36.447 : lcoffset(29)=0.0792
lcmult(30)=36.181 : lcoffset(30)=0.0804
lcmult(31)=36.105 : lcoffset(31)=-0.255
lcmult(32)=33.451 : lcoffset(32)=-0.0521

Scan (20,Sec,0,0)
Battery (batt_volt)
PanelTemp (ptemp_c,250)

'Allow for bypassing program, such as when tinkering with it or when changing load cells, etc
' and you don't want non-useful data. Flag 1 has to be "on" (lit) for program to run.
If flag(1) = true Then

'When Flag 2 is on, bypasses normal 5 cycle to get average
'good for recalibration or to check things. Needed for irrigation control.
If flag(2) = true Then
readlysimeters = on
lysloopcount=4
EndIf

'measures load cells attached to #1 AM 16-32 in Dff channel 1 and Excite channel 1, returns mass
If readlysimeters = on Then
lysloopcount = lysloopcount + 1 'if lysloopcount=4 then this runs only once
PortSet (4 ,1 )
lcnum = 0
SubScan(600,msec,16)
PulsePort(5,20000)
lcnun = lcnun + 1
'integration set to 50Hz for China
BrFull (mass(lcnun),1,mV7_5,-
1,Vx1,1,2500,False,False,34000,_60Hz,lcmult(lcnun),lcoffset(lcnun))
NextSubScan
PortSet (4,0)
EndIf

'measures load cells attached to #2 AM 16-32 in Dff channel 2 and Excite channel 2, returns mass
If readlysimeters = on Then
lcnun = 16
PortSet (6,1)
SubScan(600,msec,16)
   PulsePort(7,20000) 'mux2 clock set to control port 6
lcnun = lcnun + 1
'integration set to 50Hz for China
BrFull (mass(lcnun),1,mV7_5,-
2,Vx2,1,2500,False,False,34000,_60Hz,lcmult(lcnun),lcoffset(lcnun))
NextSubScan
CallTable meanmass
PortSet(6, 0)
lcnun = 0
EndIf

'When lysloopcount = 5, by either instance measure or 5 rep mean, shuts down load cell reading and calls
't for mean mass of each lysimeter.
If lysloopcount = 5 Then
   flag(3) = true
   CallTable meanmass
EndIf

'Retrieves average mass per lysimeter from meanmass table and puts it in array lys_mass().
If flag(3) = true Then
   GetRecord(lys_mass(), meanmass,1)
EndIf

'After average mass retrieved, resets conditions to initial values for next lysimeter read.
If flag(3) = true AND lysloopcount = 5 Then
   flag(3) = false
   lysloopcount = 0
   readlysimeters = off
If irrbypass = off Then
  flag(2) = false
EndIf
EndIf

'Reads lysimeter every half hour and stores data in table named halfstor
If TimeIntoInterval (29,30,Min) Then
  readlysimeters = on
EndIf

If TimeIntoInterval (0,30,min) Then
  CallTable halfstor
EndIf

'Stores 5 am masses for ETa and irrigation volume determinations
If TimeIntoInterval (300,1440,Min) Then
  For i=1 To 32 Step 1
    mass_500(i)=lys_mass(i)
  Next i
EndIf

'Stores 11 pm mass for irrigation volume and ETa determination
If TimeIntoInterval (1380,1440,Min) Then
  For i=1 To 32 Step 1
    mass_2300(i) = lys_mass(i)
  Next i
EndIf

'At midnight, calculates cumulative daily ETA and setup irrigation for rest of night
If TimeIntoInterval (0, 1440, min) Then
  flag(7) = true
EndIf
If Flag(7) = true Then
  For i=1 To 32 Step 1
    PEta2400(i) = mass_500(i) - mass_2300(i)
    If PEta2400(i)< 0 Then
      PEta2400(i) = 0
    EndIf
    Total_ETa(i) = PEta2400(i) + Post1pm_massinc(i)
  Next i
  '----------------------------------Correction factor ----------------------------------------------
  'try using 1.0 as a correction factor when the root volume to soil ratio is low.
  Increase this correction factor as the plant increases
  'Changing the 1.0 to <1 would constitute a slow dry down
'Or should be increased if 5 am mass declines and it is not the irrigation system's fault
'for multiple treatments, add additional 'For i=X To Y Step 1' statements
'change the 0.333 multiplier to 1 for single daily application and adjustments to code for the 3 waterings below
For i=1 To 32 Step 1
  Totalirr(i)= PEta2400(i)* 1.0
  Partirrvol(i) = Totalirr(i)* 0.333
Next i

'Sets limit for 1/3 irrigation volume to 10 kg, if reached or exceeded,' Underirrigation flag lites up
For i=1 To 32 Step 1
  If partirrvol(i) >= 10 Then 'adjust as necessary
    partirrvol(i) = 10
    flag(5) = true
  EndIf
Next i
MaxSpa (irrneeded,32,partirrvol(1)) 'modify for multiple treatments
CallTable Dailysum

'Setup for initial mass for nighttime irrigation
For i = 1 To 32 Step 1
  preirr_mass(i) = lys_mass(i)
Next i
flag(7)= false
flag(9) = true
EndIf

'Sets up for 1 am irrigation
If TimeIntoInterval (60,1440,Min) Then
  For i = 1 To 32 Step 1
    preirr_mass(i) = lys_mass(i)
  Next i
  MaxSpa (irrneeded,32,partirrvol(1))
EndIf

'Sets up for 2 am irrigation
If TimeIntoInterval (120,1440,Min) Then
  For i = 1 To 32 Step 1
    preirr_mass(i) = lys_mass(i)
  Next i
  MaxSpa (irrneeded,32,partirrvol(1))
EndIf
' Roger does not think we will need a daytime irrigation
'Sets up for midday irrigation.
'To stop midday irrigation, change flag(8) = true To flag(8) = false
If TimeIntoInterval (781,1440,Min) Then
  flag(8) = true
EndIf
If flag(8) = true Then
  For i = 1 To 32 Step 1
    preirr_mass(i) = lys_mass(i)
  Next i
  For i = 1 To 32 Step 1
    ETA_1pm(i) = mass_500(i) - preirr_mass(i)
    'Puts on 1/2 of ETA at midday. Can be reduced For slow drydown
    partirrvol(i) = ETA_1pm(i)* 0.5
    'limits midday irrigation To 6 kg. You may need To increase If trees are big
    If partirrvol(i) > 6 Then
      partirrvol(i) = 6
      flag(5) = true
    EndIf
  Next i
  MaxSpa (irrneeded,32,partirrvol(1))
  midday_signal = on
  flag(8)= false
  flag(10) = true
EndIf

'Irrigation shutdown cycle
MaxSpa (cd16status(),32,cd16cntl(1))
If cd16status(1)=run Then
  shutdownswitch = on
EndIf
If irrindicator = on AND shutdownswitch = on Then
  Call irrshutdown
EndIf

'Irrigation startup cycle
If irrneeded(1)> 0.2 Then
  startupswitch = on
EndIf
If startupswitch = on Then
  Call Irr_start
  Flag(11) = true
EndIf
solenoid control
SDMCD16AC (CD16cntl(),2,0) ' 2 replicationssmd goes to next address (1) at the end of 1
cd16status(1) = off

'Post-1 pm irrigation mass increase and cleanup
If midday_signal = on AND endirrcyc = on Then
    middaydelay = middaydelay +1
    If middaydelay = 11 Then
        readlysimeters = on
    EndIf

If middaydelay = 33 Then
    For i = 1 To 32 Step 1
        Post1pm_massinc(i) = lys_mass(i) - preirr_mass(i)
    Next i
    midday_signal = off
    endirrcyc = off
    middaydelay = 0
    irrbypass = off
    midday_signal = off
    EndIf
EndIf
EndIf
NextScan
EndProg
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Date  April 16, 2012

Name  Nisa Leksungnoen
Address  8 Utah State University, Aggie village, Apt. G, Logan, Utah, 84341
Phone  951-756-5489

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Materials and Methods in Chapter 4

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Date  April 16, 2012

Name  Nisa Leksungnoen
Address  8 Utah State University, Aggie village, Apt. G, Logan, Utah, 84341
Phone  951-756-5489

Dear  Jonathan Carlisle:

I am in the process of preparing my Dissertation in the Plants, Soils, and Climate at Utah State University. I hope to complete in the Spring of 2012.

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Nisa Leksungnoen

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Programming in Chapter 6 and Appendix 1

Signed_________________________________________________________
CURRICULUM VITAE

Nisa Leksungnoen

Personal Data

Born in Thailand, October 3, 1981

Education

2007 – 2012: Ph.D. candidate in Plant Science, Department of Plants, Soils, and Climate, Utah State University, Logan, Utah (expected 5/2012). GPA: 3.95

2003 – 2006: Master of Science, Faculty of Forestry, Kasetsart University, Bangkok, Thailand. GPA: 3.94

1999 – 2003: Bachelor of Science, with first class honors, Faculty of Forestry, Kasetsart University, Bangkok, Thailand. GPA: 3.77

Special Areas of Interest: Plant Physiology and Plant Ecology

Research Experiences

2002 – 2003: Research assistant, Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.

2004 – 2005: Teaching assistant, Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok, Thailand.
Publications


Conference poster presentations (abstracts)

