CONIFERS RESPONSE TO WATER STRESS: PHYSIOLOGICAL RESPONSES AND EFFECTS ON NUTRIENT USE PHYSIOLOGY

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A DISSERTATION

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

Forestry – Doctor of Philosophy

2019

ABSTRACT

CONIFERS RESPONSE TO WATER STRESS: PHYSIOLOGICAL RESPONSES AND EFFECTS ON NUTRIENT USE PHYSIOLOGY

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Conifer species are the most extensively distributed on earth, and they are one of the most significant renewable resources with high economic value. Conifer species, *Pinus* and *Abies* species have been gaining popularity due to their desirable green color for products such as Christmas trees and are extensively used in landscaping. Not only inhabiting forest in their natural habitat, but also in plantations and reforestation areas usually outside their natural range where they have been exposed to water stress due to water shortage and the effects of climate change.

Water stress is an important environmental factor for tree growth and development in plants. Therefore, we investigated the effect of irrigation and fertilization on balsam (*Abies balsamae*) and concolor fir (*Abies concolor*) and white pine (*Pinus strobus*) seedlings in terms of tree morphology and physiology using a factorial design with three species and irrigation levels and two fertilization rates. Increased irrigation not only increased morphological traits such as diameter and height growth but also increased the net photosynthesis and stomatal conductance. The combination of each treatments had 5 seedlings for fir species and 4 seedlings for the pine species totaling 168 individual trees. White pine and balsam fir showed some drought tolerant mechanisms where concolor fir exhibited drought avoidance mechanisms. Fir species had higher net photosynthesis, stomatal conductance, transpiration rate and a lower water use efficiency compared to white pine. White pine had lower potassium concentration compared to two fir species, and balsam fir had higher calcium concentration compared to white pine and concolor fir under stress conditions, implying that fir species are more susceptible to water stress.

We observed that concolor fir had a greater capacity for conserving water compared to white pine, leading to better above ground growth and shoot to root ratio. Balsam and concolor fir also had a greater foliar nitrogen concentration compared to white pine seedlings due to using an avoidance mechanism and maintaining nutrient uptake under water deficit conditions. White pine trees use drought tolerance strategies to reduce transpiration and maximize water uptake with increased root systems. White pine trees had higher below-ground biomass, with increased fine and coarse roots, and a lower nutrient use efficiency compared to two fir species.

Moreover, we also tested the provenance and altitudinal variation of Turkish fir seedlings under water stress conditions. Morphological traits, such as relative root collar diameter, relative height growth, and stem volume index differed with seed source altitude as transplants from higher seed sources altitudes had greater growth compared to seedlings from lower altitudes. Overall, provenance had little effect on physiological parameters such as net photosynthesis, stomatal conductance, transpiration rate, internal CO_2/ambient CO_2 ratio, water use efficiency ($WUE=A/E$) and intrinsic water use efficiency ($WUEi = A/gs$), chlorophyll fluorescence (Fv/Fm) and carbon isotope discrimination rate ($\Delta^{13}C$). Provenances varied in stem water potential and net photosynthesis. Seedlings from the Karabuk provenances had high stem water potential and net photosynthesis. Intrinsic water use increased with altitude of the seed sources as seedlings from higher altitudes showed higher values compared to lower altitudes. Karabuk provenances might be more sensitive to water availability than Adapazari provenances. Adapazari provenances should be selected for the plantation and afforestation areas and production of Christmas trees.

Dedicated to my grandfathers,
Ekrem Koç and Ahmetcan Atmaca.
You both were the first to teach me a lot about plants and their practices.
I miss you, both.

ACKNOWLEDGEMENTS

I would first and foremost like to thank my major professor, Dr. Pascal Nzokou for giving me the opportunity to work with him on this project. He did a wonderful job not only supporting and encouraging me, but also providing me with the knowledge, guidance, and support. This project has been a very good learning experience and has helped me expand my knowledge and thought process immensely. I am also thankful for committee members Dr. Bert Cregg, Dr. Sophan Chhin and Dr. Paolo Sabbatini who spent many hours reading my dissertation and also for providing guidance and help during my project.

I would like to thank the farm managers Randy Klevickas and Paul Bloese at the Tree Research Center for their patience and assistance. I am thankful for the help of my fellow colleagues Patrick Shults, Chad Papa, Dilek Yildiz, and several undergraduate assistants I worked with through the course of this project. I am sincerely grateful for the friendship and emotional support given by Salih Armagan, Cem Celal Tutum, Baburhan Uzum, Alp Ozdemir, Xinyue Cui, Nihan Çetin, Murat Uzel, Beyaz Basak Koca, Acimovic family, and all of my volleyball and soccer friends.

Most important I would like to thank you my mother, father, and parents for supporting my decision to continue my education and the support they provided for me during this whole time. Furthermore, I extend many thanks to all my family and friends who have cheered me on and supported me throughout this process. You all mean the world to me. Lastly, I would like to acknowledge the Republic of Turkey - Ministry of National Education department for the funding and financial support of me throughout years.

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CHAPTER ONE

GENERAL INTRODUCTION

Global warming is probably the biggest threat facing the world in the 21st century. One of the biggest effects of global warming is a rapidly changing climate, proven to be a consequence of the result of the rapid industrialization that occurred in the 20th century. The average temperature of the Earth has risen almost 1 °C over the past 100 years (IPCC 2014). According to IPCC (2014) the average global temperature could increase at the range of 0.8-2.6°C by the year 2050 and 1.4 to 5.8°C by the year 2100. Increasing temperature can affect and/or change ecosystems, species, and their habitats, forests and agricultural areas and cause many environmental issues including drought and water stress on plant species.

Conifers are the most widely distributed plants on earth, and they are not only one of the world's most significant renewable resources but are also very important for their economic value (Earle 2017). Amongst conifer species, *Pinus* and *Abies* species have been gaining popularity due to their desirable color for ornamental value such as Christmas trees and are widely used in landscaping (Earl 2017). Christmas tree production is of considerable commercial interest in Europe, the United States of America and Canada where the demand is high. Currently, there are approximately 25-30 million Christmas trees sold every year and almost 350 million trees currently growing in plantations in the U.S. (NCTA 2018). However, trees have been facing some environmental stresses not only in their natural habitat, but also in plantations and reforestation areas usually outside their natural ranges where they are exposed to water shortage or lack of precipitation and the effects of climate change (Grossnickle 2005).

Under plantation culture, trees are exposed to many environmental stresses such as abiotic, chemical and physiological factors that adversely affect growth, development, or productivity. However, plants including coniferous species, have some intrinsic coping mechanisms to deal with extreme conditions, which allow them to survive environmental changes (Lange et al. 2002).

Under soil water deficits (water stress conditions), plants can demonstrate either drought escape, drought resistance, drought avoidance (maintenance of tissue water potential) or drought tolerance mechanisms (Levitt 1980; Price 2002) (Table 1.1).

Table 1.1. Drought resistance mechanisms in conifers.

Type	Traits/Responses
Drought Stress Avoidance	Stomatal control, Shoot growth, Increasing rooting, Hydraulic conductance, Low stomatal conductance (*gs*), Reducing transpiration and transpiration area
Drought Stress Tolerance	Osmotic adjustment, Cellular elasticity, Accumulation of solutes (Abscisic acid [ABA], ethylene, cytokinins, amino acids, proline, ROS sugar, anions and cations), Changes in gene expression and signaling

In addition to water, mineral nutrition plays a crucial role to fulfill not only plant growth and development but also physiological functions (Groves et al. 1998). Under droughty conditions, soil water deficit alters numerous steps of water transfer along the soil-root-leaf-atmosphere continuum that also impacts nutrient uptake by plants (Bréda et al. 2006). This process is vital for plants grown under intensive management because farmers fertilize trees to accelerate growth and meet the plant’s nutrient requirements (Bilderback 1999). To alleviate the adverse effects of water stress, tree root systems play a critical role in water and nutrient transport, thus desirable tree growth and development is needed (Alam 1999).

Plant growth and development characteristics vary from species to species (Brukhin and Morozova 2011). It is well known that morphological features and biomass allocation are affected by water stress (Akinci and Lösel 2012). Several studies pointed out that, when exposed to water stress, plants develop higher root/shoot ratios than non-stressed seedlings (Grossnickle and MacDonald 2018; Lanchenburch 2016; Akinci and Lösel 2012; Zhao and Liu 2009; Granier et al. 2007; Chaves et al. 2003). However, growth and development patterns in response to water stress vary between species. Besides that, elevation gradients have a significant impact on both structural and functional plant traits (Losso et al. 2016). It is established that altitudinal plant distribution has an impact on plant growth, and seedling establishment (Kelly and Goulden 2008). In the natural distribution of species, the environmental conditions in low altitudinal areas are more vulnerable to drought due to low rainfall and high potential evaporation. Under the contrasting environmental conditions in temperate zones, population differences occurred in these species based on their tolerance to environmental stresses such as drought.

This project investigates the morphology and physiological responses of Eastern white pine (*Pinus strobus* (L.)) (MI), balsam fir ‘Cooks’ (*Abies balsamae*) (NY), concolor fir ‘Cibola’ (*Abies concolor*) (NM) and Turkish fir (*Abies bornmuelleriana*) species under water stress. In addition, I investigate the effect of fertilization on nutrient uptake and nutrient use efficiency under water deficit conditions. We also examine the effects of the altitudinal variation on growth and establishment success of Turkish fir under water stressed conditions.

Concolor fir is native to western and southern United States of America including Oregon, California, Idaho, Wyoming, Colorado, Utah, Arizona and New Mexico (Laacke, 1990) while Balsam fir naturally occurs in eastern and central Canada, and the northeastern United States reaching out to the Appalachian Mountains and West Virginia in the south (Frank 1990). Eastern white pine is native to eastern North America from Canada to Great Lakes region which extends its distribution to southeastern Minnesota in the west and Georgia and Alabama in the south of the USA (Wendel and Smith 1990). Annual precipitation ranges from about 760 to 1100mm, 510 to 890 mm, and 510 to 2030 mm for balsam fir, concolor fir and Eastern white pine, respectively. These tree species have been extensively planted in the United States of America and are of high economic importance for various intensive production systems and there are indications that they will continue to be planted in large scale afforestation programs.

I hypothesize that white pines will have a greater tolerance to water stress compared to balsam fir and concolor fir due to their ability to develop a more mature tap root system at an earlier age (Brown and Lacate 1961). Additionally, an increase in roots would provide for an increased adaptation for nutrient uptake under water stress in white pine compared to the two-fir species. I also hypothesize that seedlings obtained from seed sources originating from lower elevation would better tolerate water stress compared to plants originating from higher elevation sources.

The specific objectives of this dissertation were to:

1) Determine and compare morphological and physiological responses of white pine and fir species to water stress.

2) Determine and compare the biomass allocation and nutrient use physiology in white pine, concolor fir and balsam fir under water stress.

3) Determine the effect of altitudinal variations (elevation) on early growth and physiology in Turkish fir under water stress.

This dissertation is structured to introduce the topic and discuss the significance of the problem, then present a review of the literature on conifers and drought stress mechanism and physiology, followed by a collection of three papers (corresponding to the three objectives) that have been or will be submitted for publication in forestry and horticulture related journals. Finally, I close with a general conclusion and recommendations for future studies.

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CHAPTER TWO

LITERATURE REVIEW

2.1. INTRODUCTION

Conifers are the most distributed plants on earth, and inhabit most boreal, temperate and boreal mountain forests, across vast expanses in North American, African, European and Asian semiarid woodlands (Earle 2017) (Fig. 2.1). According to Earle (2017), conifers are one of the world's most significant renewable resources. Although the total conifer forestland is reduced as a result of deforestation and global warming, conifers are still widely distributed. Conifers occur on all continents except Antarctica, however, the conifers are well known to most of us from the widespread forest of pines and spruces in the northern hemisphere (Farjon 2018). Most conifer species are considered commercially important trees and they have been extensively harvested in Europe, North America and Canada where the demand is high.

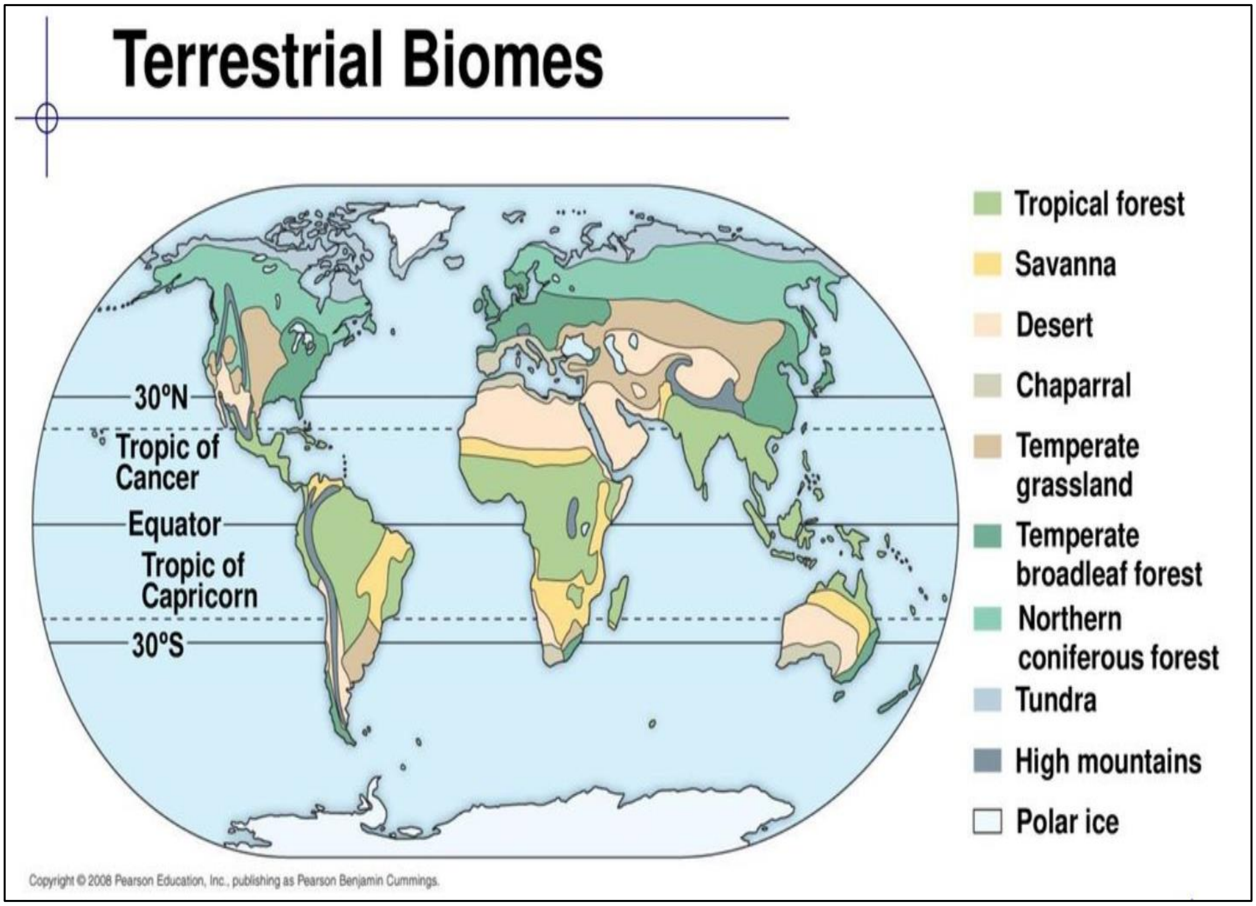

Figure 2.1. Conifer distribution in the World. Obtained from Pearson Benjamin Cummings 2006).

2.2. BIOLOGICAL AND PHYSICAL CHARACTERIZATION OF CONIFERS

2.2.1. Taxonomical Background of *Abies* and *Pinus* and Their Distribution

Conifers have 615 species and are very important in terms of economic value. *Pinus* and *Abies* genera have been gaining popularity due to their desirable color for ornamental value (Christmas trees) and are widely used in landscaping (Earle 2017). They are the most widely distributed plants on earth from North America to Africa, Europe and Asia with the exception of Antarctica (Earle 2017).

Approximately 40 to 50 species of *Abies* exist around the world (Table 2.1). North America has 9 of the 40 species, such as *Abies concolor, Abies balsamae, Abies fraseri*, *Abies grandis*, *Abies procera*, *Abies magnifica, Abies lasiocarpa, Abies grandis, Abies bracteata* that occur worldwide in the Northern Hemisphere. Taxonomists are unsure how many *Abies* species exist in intermediate form as varieties of distinct species or form hybrids between distinct species. Due to a lack of general consensus between taxonomists on the number of true fir species, the scientific classification of some fir species is confusing (Frampton 1998). For instance; it is claimed that *A. bornmulleriana*, *A. equi-trojani*, and *A. cilicica subsp. isaurica* are endemic plants (Tumen et al. 2010). It is also established that *Abies equi-trojani* is a narrow endemic species due to its unique growth forms and ability to grow faster compared to other fir species in Turkey (Kaya et al. 2008).

Table 2.1. *Abies* genera (10 sections – 46 species) separation (Farjon 1990).

Section	Species
Abies	*alba* Mill.
	cephalonica Loud.
	cilicica (ant. Et Kotschy) Carriére
	nebrodensis (Lojac.) Mattei
	nordmanniana (Stev.) Spach
Amabilis	*amabilis* (Dougl.) Forbes

Table 2.1. Cont'd.

Section	Species
	mariesii Mast.
Balsamea	*balsamea* (Linn.) Mill.
	fraseri (Pursh.) Poir.
	kawakamii (Hay.) Ito
	koreana Wils.
	lasiocarpa (Hook.) Nutt.
	nephrolepis (Tratv.) Maxim.
	sachalinensis (Fr.Schm.) Mast.
	sibirica Ledeb.
	veitchii Lindl.
Bracteata	*racteata* D. Don ex Poiteau
Momi	*beshanzeunsis* Wu
	chensiensis Van Tiegh.
	firma Sieb. Et Zucc.
	holophylla Maxim.
	homolepis Sieb. Et Zucc.
	pindrow (Lamb.) Royle
	recurvata Mast.
	ziyuanensis Fu et Mo
Grandis	*concolor* (Gord. Et Glend.) Lindl.
	durangensis Mart.
	grandis (Dougl.) Forbes
	guatemalensis Rehd.
Nobilis	*magnifica* A. Murr.
	procera Rehd.
Oiamel	*hickeli* Flous et Gauss
	religiosa (H.B.K.) Schlect. et Cham.
	vejari Mart.
Piceaster	*numidica* De Lann.

Table 2.1. Cont'd.

Section	Species
	pinsapo Boiss.
	densa Griff.
	fabric (Masters) Craib
	fanjingshanensis Huang, Tu et Fang
	fargesii French.
	forrestii C. Coltm. Rogers
	spectabilis (D.Don) Spach
	chengii Rushforth
	delavayi Van Tiegh.
	squamata Masters
	yuanbaoshanensis Lü et Fu

The *Pinus* genus is the largest and oldest in the conifers' family that includes more than a hundred species (Table 2.2) and not only grows in the Northern hemisphere but also survive in different areas with temperate and subtropical climates (Earle 2017). The hard and soft pines are the two subgenera of the conifers which include about 70 and 44 species respectively (Richardson 1998). Most pines can grow on acidic well-drained soils and up to 5200-meters altitude (Earle 2017). Pine trees can reach astonishing heights in their natural habitats, ponderosa pine, for example, reaches heights of 30 to 50 meters (Wennerberg 2004b). Pine trees are characterized by having needles and cones and each tree needs its own description due to physical diversity within the genus (Earle 2017). Richardson (1998) stated that hard pines have two leaf vascular bundles per needle, each fascicle has 2-6 needles, and stomata are more or less uniformly spread on all surfaces. On the other hand, white or soft pines have one leaf vascular bundle per needle, 1-5 needles each fascicle, and stomata are mostly on inner faces (Richardson, 1998). Needles are like on foxtail pine and the minimum length can be less than 2-3 cm and the maximum length can reach

Table 2.2. *Pinus* genus (111 species) as defined by Gernandt et al. (2005).

Subgenus	Section	Subsection	Number of Species	Distribution	Well-known species
Pinus	*Pinus*	*Pinus*	17	Eurasia	*P. sylvestris, P. kesiya*
				North America	*P. merkusii*
	Trifoliae	*Pinaster*	7	Mediterranean, Asia	*P. sylvestris*
		Contotae	4	America	*P. banksiana, P. contarta*
		Australes	26	America	*P. elliottii, P. radiata*
		Penderosae	17	America	*P. jeffreyi, P. penderosa*
Strobus	*Parrya*	*Balfourianae*	3	America	*P. balfouriana*
		Cembroides	11	America	*P. cembroides* *P. culminicola*
		Nelsoniae	1	Central America	*P. nelsonii*
	Quinquerfoliae	*Gerardianae*	3	Asia	*P. burgeana* *P. gerardiana*
		Krempfianae	1	Asia	*P. krempfii*
		Strobus	21	America, Eurasia	*P. cembra, P. lambertiana, P. strobus*

around 60 cm (Earle 2017).

2.2.2. Morphology and Physiology of Fir and Pine Species

Firs may grow to be between 20 to 80 meters tall in the wild; they differ from other species of pines because of their different shape, bark, and scent. They have a white line below their foliage and the foliage is a blue-gray color, similar to Colorado blue spruce (Frampton 1998). Firs are evergreen and have smooth and plump trunks that have a tapering root system. However, they are also able to establish a lateral root system (General Directorate of Forestry 2010). Their cone is held upright mostly on the upper branch which differs from *Picea* species (Edwards 2008). Fir needles are soft, flat and cannot be rolled between fingers, unlike spruce species.

Pine trees are long-lived trees with a thick and scaly bark, and the trees and shrubs of pines are grown 3-80 meters tall, mostly 25-45 meters tall. Pines are mostly monoecious (rarely semi-dioecious) in which the male and female cones occur on the same tree (Earle 2017). Hard pines have 2-6 needles for each fascicle and two leaf vascular bundle per needle while soft pine has 1-5 needle each fascicle and a single vascular bundle per needle (Richardson 1998). In general, cones take a couple years to mature and cones may have wood scales and be stiff (Earle 2017). Most pine species continue a dominant role in forest successions due to being very drought tolerant and fire adapted (Earle 2017).

Conifer species have tracheid which are relatively inefficient for water transport from soil-plant-air continuum, but allows for water storage (Smith and Hinckley 1996). Stomatal conductance controls the transpiration in conifer species in that conifer species show lower transpiration rates compared to broadleaved trees (Smith and Hinckley 1996). In addition, growth rate, temperatures changes, photosynthesis, nutrient uptake, and tree responses to the environmental stress factors change among conifer species in areas with low mean annual temperatures compared to warmer areas (Smith and Hinckley 1996).

2.2.3. Morphology and Physiology of Model Species for this Study

Concolor fir species (*Abies concolor*) (or white fir) grow up to 60 m tall and 190 cm diameter at breast height and have longer blue needles as blue as a blue spruce (Earle 2017). It is native to the western United States and grows 30 to 60 cm per year in favorable places (Earle 2017). Concolor fir species do not grow in wet sites and are affected by frost in the spring due to early bud break bud, but they can however handle alkali soils unlike other fir species (Cregg 2009). Concolor fir is a native evergreen conifer species for USA, having a pyramidal crown and can live for over 300 years (Earle 2017) (Fig. 2.2). Concolor fir has a deep bluish-green needle color which is 3-7 cm long and generally curved upward. Concolor fir is considered a moderately drought tolerant species.

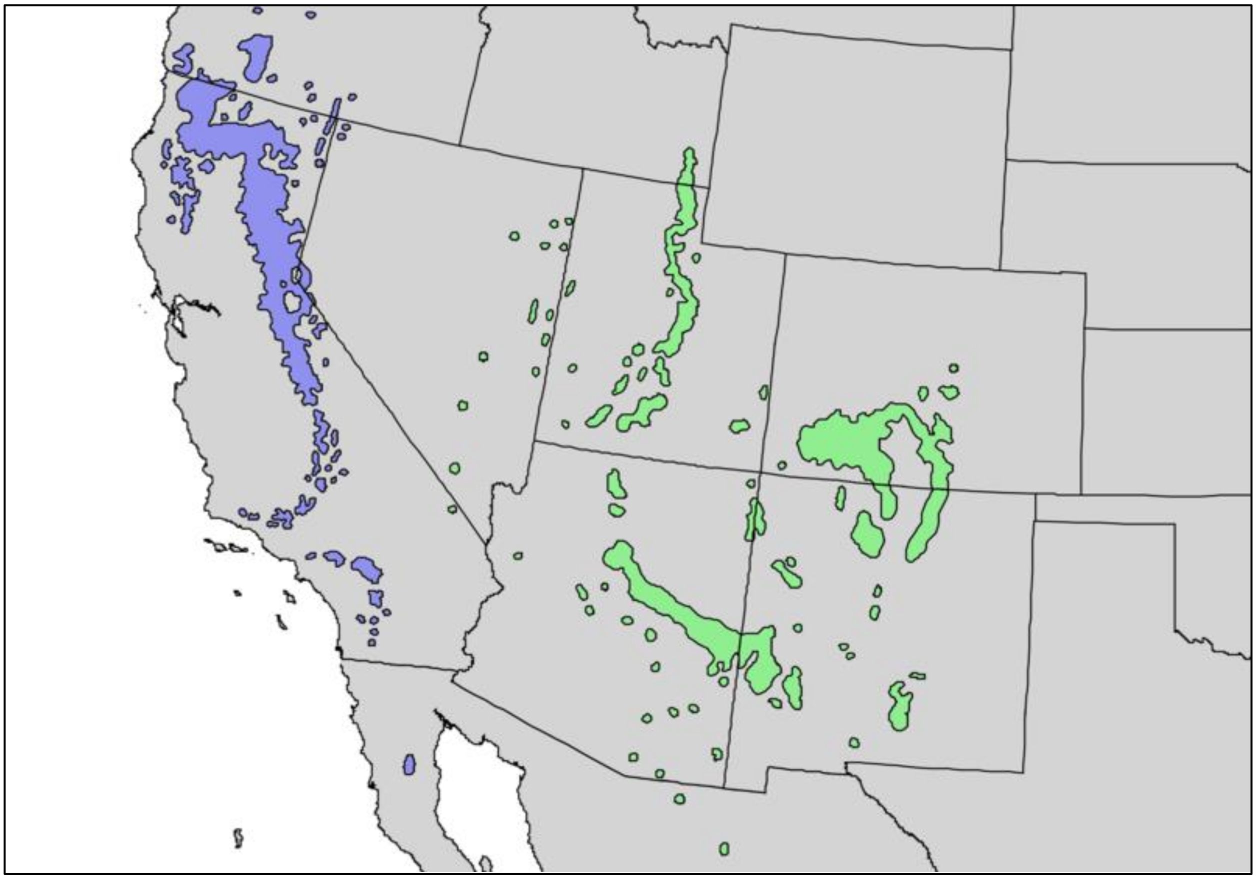

Figure 2.2. *Abies concolor* distribution in North America (Laacke 1990).

Balsam fir (*Abies balsamea*) is native to most of eastern and central Canada and the northeastern United States (Fig. 2.3). Balsam fir has two varieties; *Abies balsamae* var. *balsamae* and *Abies balsamae* var. *phanerolepis*. Although, balsam fir is a slow growing tree species, it has been so popular species for customers due to the strong scent with dark green needles and excellent form of Christmas tree (Cregg 2016). Balsam fir can grow up to 12-23 m tall and 10-60 cm diameter at breast height (Earle 2017). The leaves are flat and needle-like, 1 to 3 cm long, two white stomatal bands below and dark green color above (Earle 2017). They have a shallow root system which can reach up to 75 cm, and it has a low drought tolerance.

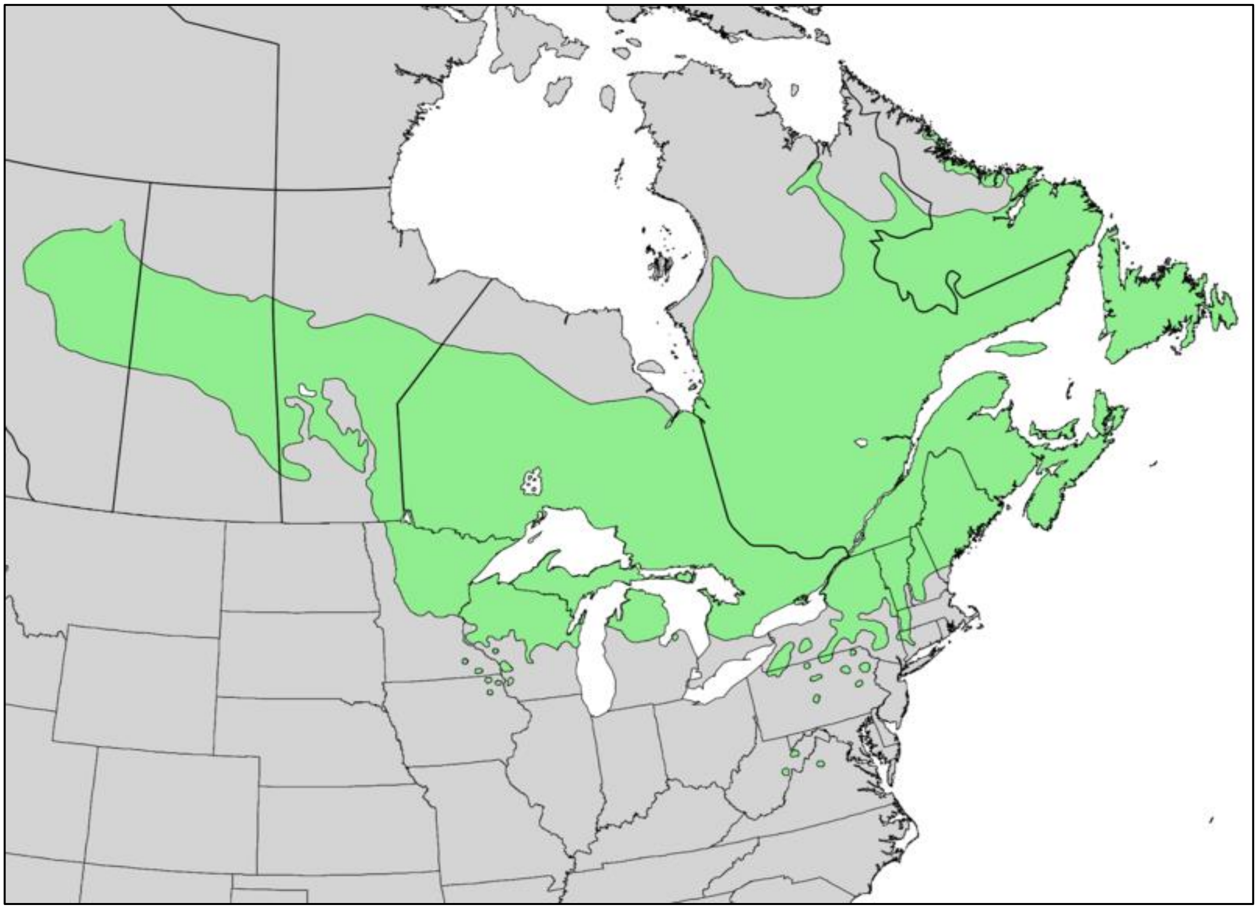

Figure 2.3. *Abies balsamea* distribution in North America (Frank 1990).

Abies siberica (to Baikal Lake in the east) and a number of other species (*A. nephrolepis*, *A. sachalinensis*, *A. holophilla*) found in far eastern Russia (Shvidenko et al. 2007). Four fir species

A. nordmanniana, *A. bornmülleriana*, *A. cilicica*, and *A. equitrojani* are naturally located in Turkey (General Directorate of Forestry 2010). *Abies nordmanniana* is also distributed in West Caucasia (Abkhazia and Georgia) (Edwards 2008). *Abies bornmülleriana* Mattf. (Turkish Fir): The Turkish fir tree species are indigenous to Turkey, and it is found from Kızılırmak Valley in northern Anatolia to Uludag in the west (General Directorate of Forestry 2010) (Fig. 2.4). It is claimed that Turkey fir is the hybrid of Grecian × Turkey fir hybrid (*A. equi-trojani*) (Edwards 2008).

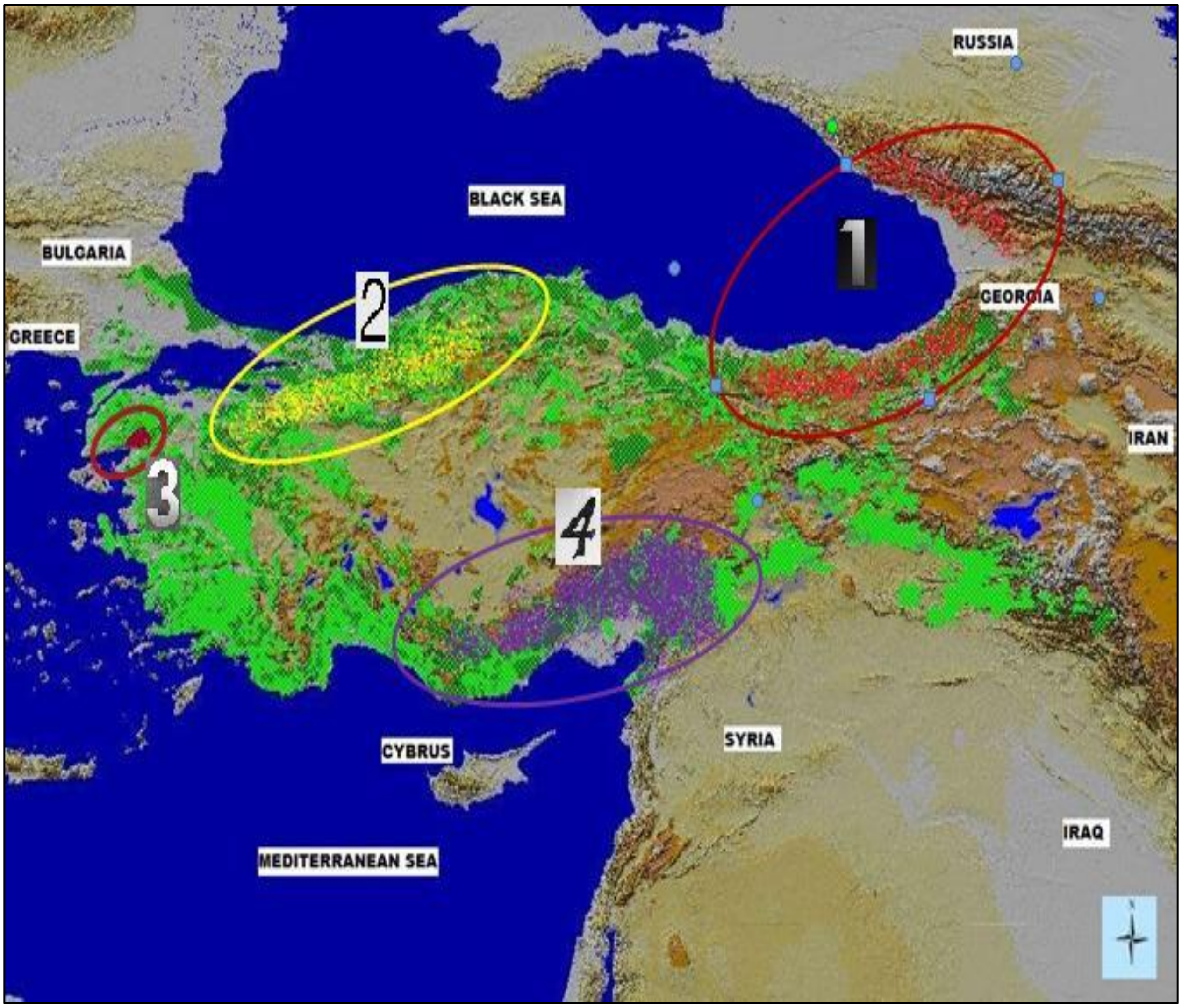

Figure 2.4. Fir species distribution in Turkey. Numbers are represented to one species as followed. 1- *Abies nordmanniana*; 2- *Abies bornmülleriana*; 3) *Abies equi-trojani*; 4) *Abies cilicica* (General Directorate of Forestry, 2010).

Pinus strobus (L.), commonly called Eastern white pine, is a fast-growing softwood, and long-lived evergreen tree that is distributed throughout much of eastern North America (it is the State tree of the States of Maine and Michigan) (Wendel and Smith 1990) (Fig. 2.5). Eastern white pine typically grows approximately 30 meters tall in the wild and is not only important for ecological purposes (reforestation) but is also economically important due to its exceptional wood properties and landscaping and Christmas trees (Wendel and Smith 1990). They are easily grown in fertile soils and cool, humid climates such as medium climates, well-drained soil in full sun.

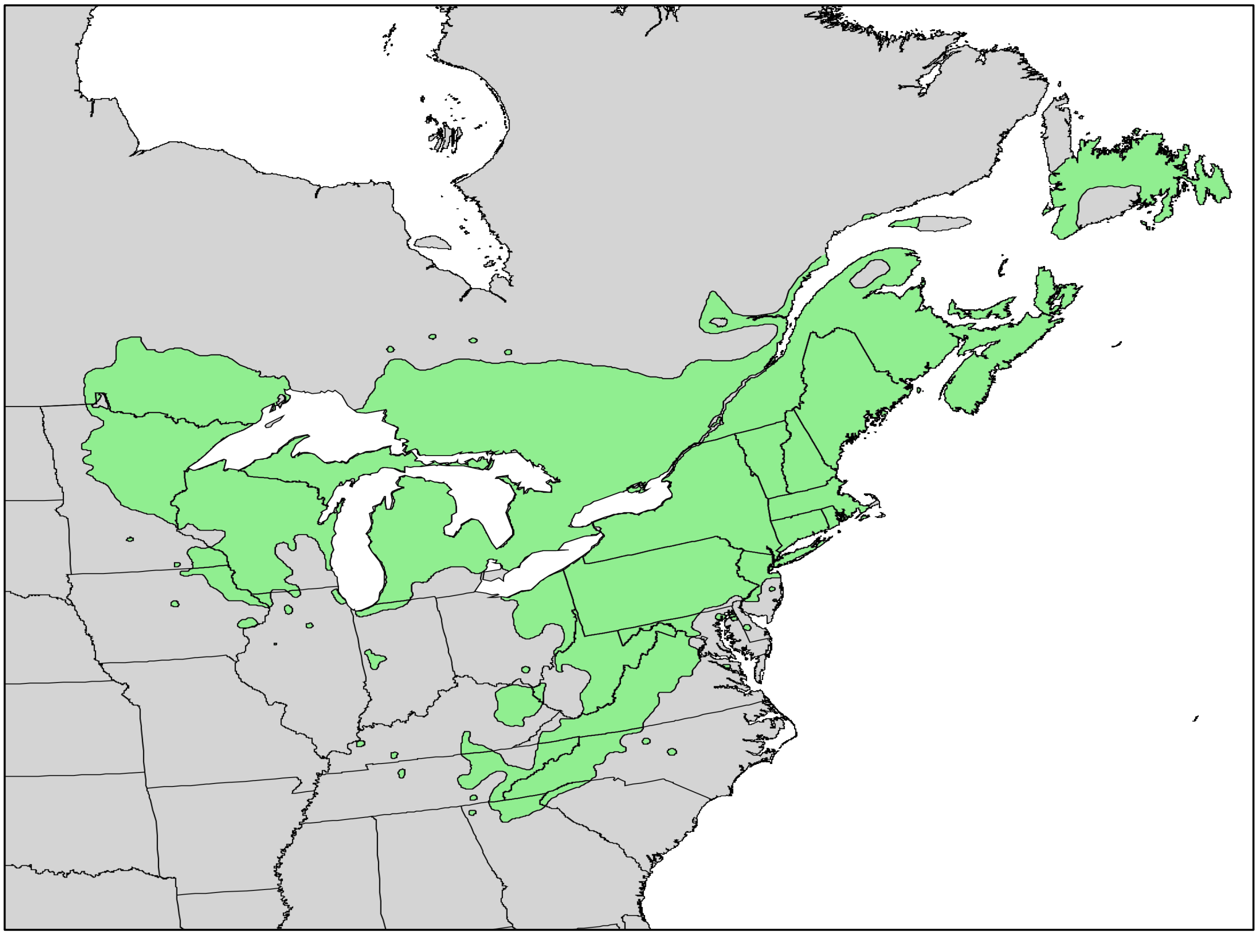

Figure 2.5. *Pinus strobus* (L.) distribution in North America (Wendel and Smith 1990).

2.2.4. Ecological and Economic Importance of Conifer Species

Conifer trees are important ecologically in that they make up the majority of the biomass in Northern hemisphere and are major sources of primary production to various ecosystems. They are also a carbon sink to sequester carbon from atmosphere. Also, conifer species are the main provider for softwood timber production, providing immense economic values. Conifer species have global importance of their usage in various areas, such as paper production, Christmas tree and food production for humans and animals. Conifer species such as pine and fir species not only provide wood products, but also are crucial in maintaining biodiversity and sustainability of forest ecosystems (Smith and Hinckley 1996).

Beside timber production from most fir species, several are used as in Christmas tree production systems. These include Fraser fir, Douglas fir (not a true fir), Balsam fir, Concolor fir, Noble fir, Korean fir, and Canaan fir are the main species in various parts of the United States of America; Silver fir, Noble fir, Douglas fir, Nordmann fir, and Turkish fir in most European production systems.

The wood of most firs generally is light and soft, and although firs are unsuitable for general timber use, firs are used to produce furniture, wooden panels, cornices, coating sheets, and building materials. Firs are also used to produce boxes, cage packages, cases, barrels, toys, etc. (General Directorate of Forestry 2010). Some firs are valued for their beauty and fragrance, and most of them are used as a Christmas tree in Europe or for considerable commercial interest (Landgren 2016) or ornamental trees for recreational works (Sevik 2012). Also, some fir and pine species are grown commercially as a Christmas tree species in the United States: such as Balsam fir, Concolor fir, Fraser fir, Eastern White pine, Scots pine…etc. In addition, conifers, such as pine and fir species provide habitat and food for many animals (Earle 2017; General Directorate of Forestry 2010). Turkish fir (*Abies bornmuelleriana*) is used for recreational works and preferred as a Christmas tree, and used for its wood that it is similar to Nordmann fir. Young shoots of subsp. *isaurica* is collected for its oil (Bağci et al. 1999) which is used in folk medicines for colds, stomachaches, antibacterial and antifungal activities, and perfumery. The wood of white pine is light and durable which is good for boxes, toys and cabinets (Dickerson 2002). White pine is used in Christmas tree plantations and as landscaping. The seeds of white pine is eaten by many animals (Dickerson 2002). White pine is also used for windbreaks and screens for roads (Dickerson 2002).

2.3. TREE RESPONSES TO ABIOTIC STRESSES

Temperature, humidity, light intensity, the supply of water and minerals, and CO_2 are the principle abiotic environmental stress factor which determine the growth and development of plants (Lange et al. 2002). Several stress factors affect plants at the same time, this is referred to as multiple stressors (Schlesinger et al. 2016; Birchler 1997).

Taiz and Zeigler (2006) state that stress is an external factor that has harmful effects on the plant. External factors can be sunlight, water, carbon dioxide, oxygen, and mineral elements from the soil that are crucial for plant growth. If the amount of these things are deficient or more than is needed, plants may become stressed and even die (Lange et al. 2002). However, plants have adapted to many extreme conditions and plants can tolerate environmental change. If the stress factor is severe enough, it will exceed the tolerance range and plants will suffer stress (Smith et al. 2001). Stress plays a crucial role in plant species distribution due to soil and climate limitations (Taiz and Zeiger, 2006).

2.3.1. Water or drought stress

One of the most severe abiotic factors limiting plant growth is drought (Terzi and Kadioglu 2006). Drought is defined as a period without significant precipitation, and drought stress can occur when there may be enough water in the soil but too much water is lost due to some atmospheric conditions, such as transpiration or evaporation (Jaleel et al. 2009). Conifers are distributed in large areas all over the world, including some of the most extreme temperature regions (Earle 2017). Many different species of conifers are faced with drought stress in their lifespan (Earle 2017), and it has been observed in Norway spruce, Scots pine, Fraser fir (Nzokou and Cregg 2010a), *Pinus ponderosa* (Dougl.) Lawson (Kolb and Robberecht 1996) and *Pinus nigra* (Arn.) (Martín-Benito et al. 2008).

Reduced water content, diminished leaf water potential and turgor loss, closure of stomata and decline in cell enlargement and growth are some of the characteristics of drought stress. These stress signs may vary from species to species, and even within a species (Jaleel et al. 2009). Severe water stress may finally result in the death of the plant due to reduced photosynthesis and the disturbance of metabolism (Jaleel et al. 2009; Nzokou and Cregg 2010b). Stomatal closure negatively affects the CO_2 assimilation and net photosynthesis (Klooster et al. 2010), and limits plant growth (Terzi and Kadioglu 2006). Drought stress can cause stomatal closure and limitation of gas exchange due to a decline in water content (Jaleel et al. 2009). Water plays a crucial role during drought, and a plant will resist drought better if it has more water. Photosynthesis, respiration, carbohydrate levels, and growth promoters are affected by water deficiency, which reduces plant growth. Water stress limits cell expansion and cell growth because of low turgor pressure. Leaf growth, and in turn the leaf area, in various species is reduced by water deficiency stress (Jaleel et al. 2009), and this not only decreases turgor but also diminishes wall extensibility and enhances yield threshold. Furthermore, water stress limits leaf number due to reduced growth rate of branches. Development of the root system is affected by mild water stress (Taiz and Zeiger 2006). Although the deep root system increases water uptake and maintains the osmotic pressure, the root dry weight decreases under mild and severe water stress. When drought stress combines with heat stress it is very destructive (Wahid et al. 2007), so much so that some plants have developed adaptation in response to water deficiency and drought, such as reduced leaf area and deeper root growth (Taiz and Zeiger 2006).

Plants are capable of responding and adapting to drought stress. The first response against drought is reducing the leaf area. Deeper root growth is another defense mechanism against drought. Stomatal closure can follow these two defense mechanisms against drought (Taiz and Zeiger 2006). Antioxidant enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR), catalase and peroxidase, and low-molecular antioxidants such as ascorbic acid, and glutathione may be an important factor to help plants tolerate environmental stress. Glutathione reductase also defends against desiccation and drought (Terzi and Kadioglu 2006). Crop plants reduce biomass production in response to water stress (Jaleel et al. 2009) ,and drought can also lead to altered pigment concentration, thus the photosynthetic apparatus can be damaged by a permanent water-deficit (Terzi and Kadioglu 2006). Photosynthetic pigments [chlorophyll (chl) a and b] are crucial for plants and can change with drought. However, carotenoids moderately help plants resist drought (Jaleel et al. 2009). The content of photosynthetic pigments (chl a, chl b, and carotenoids) is reduced during drought stress. When water becomes available, the photosynthetic system can recover. Synthesis of starch and sucrose in stressed plants helps in response to drought (Anjum et al. 2011). Plants may accumulate osmolytes such as sugar, sugar alcohols, and proline which may contribute to the plant’s response to drought stress (Wahid et al. 2007). There is a lot of research that has been conducted showing that proline content was elevated when there is an increase in the water deficit (Mohammadkhani and Heidari 2008).

2.3.2. Heat stress

In most environments, the ideal temperature range is between 5-25 °C, and it is usually necessary for the normal function of most plants (Larcher 2005). Temperature extremes at both ends of the spectrum can cause problems for plants. High temperatures (above 45 °C) cause heat stress, and plants are unable to continue to thrive under these conditions (Taiz and Zeiger, 2006). Taiz and Zeigler (2006) pointed out that heat stress initially kills the actively growing tissues, however some of the tissue may survive exposure to high temperatures. In other words, some plants are able to tolerate high temperatures at some certain levels (60 - 65 °C), however others cannot; they can get damaged due to high temperatures above 45 °C. When temperatures exceed the threshold, permanent damages can happen (Larcher 2005).

Under high temperatures, not only cell structure and cellular functions become destroyed suddenly but also protoplasm. Plants are able to stay alive until the high temperatures damage the protoplasm (Larcher 2005). According to Larcher (2005) these damages happen gradually and can be permanent depending on the threshold temperature. However, plants have evolved some mechanisms to respond or adapt to high heat stress, such as keeping their stomata closed during the day, inhibit photosynthesis and respiration, reflective leaf hairs, leaf rolling, decrease growth rate, and produce protective proteins (Taiz and Zeiger 2006). According to Taiz and Zeiger (2002), when the temperature rises 5 to 10 °C relative to mean temperature, heat shocked proteins (HSPs) are produced by plants to respond to heat suddenly, and then they stay in the cells of the plants. According to Krishna (2003), heat shock response is called a "general" cellular stress response, because a sudden change in temperature triggers a stress response found in all organisms. Heat shock genes are responsible for responding to heat stress. Heat shock genes encode the proteins that enable a plant to stay alive in a high heat situation by two general strategies; HSPs work against protein denaturation and aggregation, and target nonnative proteins for degradation, hence they assist plants to survive not only over temperature extremes but also under conditions of harsh heat stress whereby fatal temperatures can be tolerated for short periods (Krishna 2003).

2.3.3. Cold Stress (Chilling and Freezing)

Chilling injury (less than 20 °C) and freezing injury (less than 0 °C) are called cold stress and can adversely affect a plants ability for development and yield (Lange et al. 2002). If plants grow in a temperate zone and the temperature is cooled to 10 to 15 °C, growth may be getting slower, and leaves may look soggy because of chilling injury (Taiz and Zeiger 2006). According to Lange et al. (2002), the reduction of yield is approximately 40% in temperate areas. Cold stress not only affects crop productivity and quality (Larcher 2005), but also affects vegetative and reproductive stage of the plant life cycle (Nishiyama 1995). According to Beck et al. (2004) chilling injuries damage biomembranes, which lose their functions due to decreased fluidity and slowed pumps of the membrane-bound, and also energy dissipation delayed, which leads to drastic formation and oxidative stress. Under freezing dehydration, protoplasts shrink due to extracellular ice formation, adverse turgor, changes in membrane potentials, and the breakdown of membrane bilayer (Beck et al. 2004).

Cold stress can also injure the roots, trunk, and buds (Fennell 2004). If the cold stress affects the roots, the trees may wilt and ultimately die (Taiz and Zeiger 2006). Low temperatures can cause tissue dehydration when cellular water freezes (Beck et al. 2004). Even though chilling or freezing injuries can create critical disorders for plants, some have evolved responses to these injuries. According to Taiz and Zeiger (2006), genetic adaptation is one of the most important response mechanisms to chilling temperatures that can enhance chilling resistance. When chilling injury occurs, photosynthesis, respiration, and protein synthesis are decelerated, and existing protein degradation is increased due to the membranes losing their functions that resulted from changes in membrane fluidity (Taiz and Zeiger 2006).

2.3.4. Salinity stress

Salt stress may be the most common chemical stress (Larcher 2003) limiting establishment, growth, and development of plants (Evelin et al. 2009; Mathur et al. 2007). If irrigation water is inadequate, salts will accumulate from reduced leaching, this is exaggerated in arid climate zones where irrigation water is often lacking (Hamdy 2005). Unavailable drainage systems in arid areas with irrigated agriculture eventually result in salt accumulation (Taiz and Zeiger 2006). In the past, salinity was not a major issue, but today it has become a major problem to plants where irrigation water is quite limited. Some negative effects of salinity stress on plants include, physiological drought, imbalance in nutrient compound/extreme toxicity, and break down of cell organelles and their metabolism (Evelin et al. 2009).

Drought is one of the primary negative effects on plant growth and development, which results in the reduction of osmotic potential in the soil solution because of the lack of water in soil. However, plants are able to prevent water movement adversely (from roots into the soil) to sustain internal osmotic potential (Jahromi et al. 2008). Second, due to not enough water in the area, accumulated salts get more toxic, and the effects of Na^+ and Cl^- ions can move into to the cell. This then causes the breakdown of enzymes, damage to the plasma membrane and organelles, and negatively impact photosynthesis, respiration, and protein synthesis (Feng et al. 2002). Furthermore, due to the nutrient imbalance, a disruption to nutrient uptake and transportation to the shoots of the plant occurs, and the plant cannot absorbs ions from the soil (Adiku et al. 2001). Overall, salt stress can decrease photosynthetic efficiency; gas exchange, water status, and membrane break down (Evelin et al. 2009), however plants use several strategies to help mitigate salt stress (Taiz and Zeiger 2002).

Some plants (halophytes) evolved excellent response mechanisms to resist salt stress, but some of them (glycophytes-sensitive, non-halophytes-very sensitive) are not. According to Taiz and Zeiger (2006), responses to the salt injury are very similar to water stress by exclusion of excess ions from leaves or shoots. However, to reduce salt stress, plants use many strategies such as prioritized and non-prioritized organs and tissues (root exclusion mechanism), modulating ion homeostasis (ion exclusion and compartmentalization-inclusion), and metabolic adjustments (osmoregulation and compatible solutes).

2.4. PLANT WATER STRESS DRIVING FORCES

One of the most severe abiotic factors limiting growth and development of terrestrial plants is drought (Terzi and Kadioglu 2006). Drought is a meteorological term and is usually defined as a period deprived of significant precipitation. Drought or water stress appears when the available water in the soil is diminished and atmospheric conditions cause continuous loss of water by evapotranspiration (Jaleel et al. 2009).

Water transport is not only driven by the difference in water potential between the atmosphere and the substomatal cavity, but also by transpiration (Tyree and Ewers 1991). Stomata play a huge role in the regulation of water loss and water transport which are the main organs of gas exchange between the atmosphere and the plant (Sperry et al. 2002). Transpiration is elevated if the stomatal conductance is not respectively reduced throughout the high vapor pressure deficit (VPD). When plants occur in favorable conditions, water potentials are close to zero at the plant-atmosphere interface. Thus, the driving force for water transportation through the xylem is also low. However, under the dry environmental condition with dry soil, the water potentials are negative that consequently causes embolisms in the xylem conduits (Manzoni et al. 2013; Tyree and Sperry 1988). These embolisms can clog up the water column in the xylem which may cause some negative effects on plants such as shedding of foliage, and if severe enough can cause mortality (Urli et al. 2013).

Transpiration (*E*) is a driving force for water transport in soil-plant-air continuum, and it is closely associated with CO_2 uptake. When stomata are open, CO_2 enters the leaf that water is lost in the meantime. Increases in the stomatal aperture increases the water lost from stomata which is affected by turgor pressure. The turgor pressure of the guard cells and subsidiary cells surrounding the stomata are the control mechanism for opening and closing of stomata. Under favorable conditions, guard cells become fully turgid while they become gradually flaccid under water deficit (stress) that leads the plant to close stomata. Stomatal closure may result due to desiccation, regulation of osmotic solutes and accumulation of abscisic acid (Hsiao 1973; Kozlowski and Pallardy 2002). When plants conserve water to reduce risk of embolism, they also keep maintenance functions such as photosynthesis (*A*) at the same time (Brodribb et al. 2007). In general, there are two types of water management strategies that have evolved in plants: isohydry and anisohydry in which stomatal responses to water availability is linked with these two strategies. Anisohydric plants typically keep their stomata open which leads to transpiring under relatively negative water potential. Isohydry plants, however, maintain a constant midday water potential by keeping stomata closed at relatively moderate water potential (Brodribb et al. 2014) which limits transpiration. Water potential is another concept of regulation of water transport in a soil-plant-air continuum which has a huge effect on plant's sensitivity to soil moisture (Martinez-Vilalta et al. 2014).

Water stress not only affects the transport of water from the soil to the leaves, but also changes stomatal behavior in leaves and induces embolisms in the xylem. Being an anisohydric or isohydric species show some different characteristics under water stress. Anisohydric species may continue to photosynthesize while losing water through transpiration and decreasing water potential that it may cause the risk of hydraulic failure or embolisms. Isohydric species, however, close their stomata under water stress great enough to cause excessive decreases in plant water transport through the xylem.

Water use efficiency (*WUE*) refers to the rate of carbon gain (biomass or fixation of CO_2) per water lost (Sinclair et al. 1984). Water use efficiency is expressed in two ways, the first one refers to the percentage among the gain in biomass (above-ground) and loss of water (transpiration and/or evapotranspiration) during the production of that biomass. The second, photosynthetic water-use efficiency (WUE=*A/E*), refers to the ratio between carbon gain in photosynthesis and water loss in transpiration. Instead of using *A/E*, intrinsic water use efficiency (WUEi=A/g_{wv}) can be used that refers to the ratio of *A* and leaf conductance for water vapor (Comstock and Ehleringer 1992). However, there is not only a difference in intrinsic *WUE* due to the way stomata are controlled, but there is also a good correlation between photosynthetic *WUE* and *WUE* of productivity (Lambers et al. 2008). Lambers et al. (2008) indicated that plants tend to maximize *A* while minimizing *E* under the high leaf conductance (g_{wv}), *A* no longer increases linearly with intercellular CO_2 concentration (*Ci*). However, increasing leaf conductance for water vapor keeps increasing *E* due to water vapor gradient and not related to *A* (Lambers et al. 2008). Increasing leaf conductance for water vapor reduces intrinsic water use efficiency.

2.5. PLANT WATER STRESS RESPONSE MECHANISMS

Water plays a profound role in a number of plant life processes ranging from photosynthesis to nutrient uptake. Plants are exposed to the deficit of water due to some natural disasters and different environmental and physiological conditions (Akinci and Losel 2014). Drought (water stress) is one of the major abiotic factors limiting plant growth and development (Terzi and Kadioglu 2006). In general, water stress occurs when the available water in the soil is low and atmospheric conditions cause permanent loss of water by transpiration or evaporation (Jaleel et al. 2009). Water stress changes the physical environment for plant growth as well as the plant physiology (Kramer 1980). However, plants have several water stress response mechanisms, we can separate them into three groups: morphological, physiological and biochemical responses (Bohnert et al. 1995).

There are some terms related to water stress that are needed to be well-understood before we explain the response mechanisms. 'Drought resistance' is a broader term that includes escape, avoid, or tolerate mechanisms in plants (Levitt 1980). The escape strategy involves a plant structuring its life cycle, such as complete growth and reproduction phase before the stress affects plants when environmental conditions are favorable. Rapid growing, flowering, and setting seed are some examples of escape strategy. The ability of plants to keep advanced tissue water content despite a reduced water content in the soil is called 'drought avoidance' (Levitt 1980) (Fig. 2.6). Plants have two main traits involving the reduction of water loss (water savers) and improving of water uptake (water spenders). Plants that are water spenders' increase their root and hydraulic conductance to achieve higher water status while water savers reduce the loss of water by stomatal control, reducing transpiration and the transpiration area under drought stress (Basu et al. 2016). 'Drought tolerance' is the ability of plants to withstand low tissue water content through adaptive traits (Basu et al. 2016) (Fig. 2.6) which includes osmotic adjustment, cellular elasticity, and increasing protoplasmic resistance (Morgan 1984).

Conifers are the most distributed plants on earth and live in most boreal forests, most temperate and boreal mountain forests, and vast expanses on North American, African, European and Asian semiarid woodlands (Earle 2017). Conifers have adaptations to respond to the stress (Taiz and Zeiger 2006). Although each stress factors imposes different challenges in terms of metabolic and physically, plants have some tolerance mechanisms with specific components to response stresses (Sung et al. 2003).

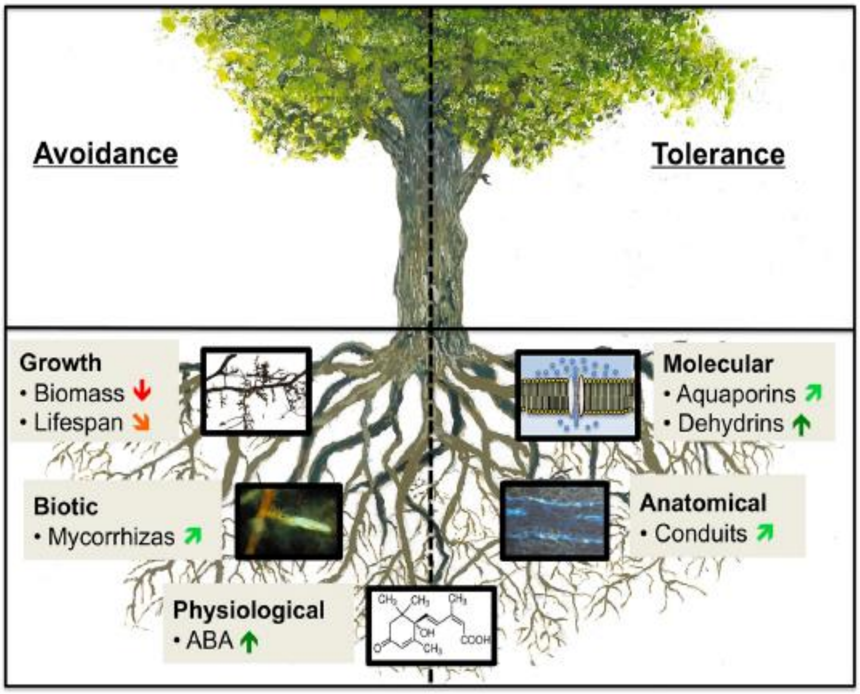

FIGURE 1 | Mechanisms of drought resistance and selected examples of tree root traits that respond to drought with avoidance or tolerance. ↑ indicates a positive effect, ↗ indicates a predominantly positive trend, ↓ indicates a negative effect and ↘ indicates a predominantly negative trend. Categories of tree root traits correspond to those given in **Table 1**. ABA, abscisic acid.

Figure 2.6. Mechanisms of drought resistance and selected examples drought avoidance or tolerance mechanisms. (From: Brunner et al. 2015).

2.6. PLANT RESPONSES TO WATER STRESS

2.6.1. Morphological responses

2.6.1.1. Growth and development

A complete understanding of processes involved in plant growth, development and productivity are required to inform better practices, especially under stress factors. The performance of plant growth and development under variable abiotic stress conditions will be dependent on the function of sources and sink limitation of the two main organs of a plant, the root system and the shoot (Anjum et al. 2011). Not only permanent but also a temporary shortage of water restrains plant growth and development more than any other environmental factors. Low germination rates leading to poor plant establishment is one of the first and foremost negative effects of water stress (Harris et al. 2002). The reduction in turgor pressure hampers cell growth, which is considered one of the most drought sensitive physiological processes (Anjum et al. 2011). Water flow from xylem to surrounding elongation cells interrupts the cell elongation of higher plants under high water deficiency (Nonami 1998). Leaf growth, the number of leaves per plant and the individual leaf size and in turn the leaf areas in various species are reduced by water deficient stress (Jaleel et al. 2009). The reduction in fresh and dry biomass production is caused by water stress in plants (Zhao et al. 2006).

Under the lack of soil water conditions, water loss can be restricted by stomatal closure which also limits not only shoot growths, but also increase root-to-shoot ratio (Brunner et al. 2015). Higher root-to-shoot ratio and deeper root systems are the main adaptive strategies for tree species under water stress (Kozlowski and Pallardy 2002) both in temperate and tropical regions (Mokany et al. 2006). The severity of stress plays a crucial role of growth parameters that the root-to-shoot ratio did not differ much under moderate water stress while the ratio shows a reasonable increase under severe stress (Brunner et al. 2015). Although trees respond to water stress by increasing root-to-shoot ratio, the biomass of fine roots and root length are mostly reduced due to decreased transpiration and respiration rates (Brunner et al. 2015).

2.6.2. Physiological responses

Plant species possess various physiological responses to water stress, including drought escape, drought resistance and drought avoidance (maintenance of tissue water potential) and drought tolerance (Price 2002; Levitt 1980). Drought escape is the first response mechanism, which is described as the ability of plants to complete the life cycle before stress sets in. Drought avoidance is the upkeep of higher water potential despite a soil water shortage. Improved water uptake, the capacity of holding acquired water, and reduced water loss are some of the drought avoidance mechanisms. Plants that have the ability to withstand against water deficit with low tissue water potential are considered drought tolerant (Price 2002). Plant species are affected differently by water stress (Akinci and Losel 2014) due to varying ability to acclimate to, and survive, the variation on hydraulic characteristic and patterns of water use (Mcdowell et al. 2008; Allen et al. 2010). The physiological responses to water stress are separated by recognition of root signals, loss of turgor and osmotic adjustment, osmolyte accumulation, accumulation of plant hormones (abscisic acid, ethylene, anions and cations…etc) reduced leaf water potential, a decrease in stomatal conductance to CO_2, reduced internal CO_2 concentration, a decline in net photosynthesis and reduced growth rates.

2.6.2.1 Root signaling

A well-developed root system is favorable to support plant growth and obtain more water from the soil during the early plant growth stage which is otherwise easily lost by evaporation. In general, when the limited water exists in the soil, the root-to-shoot ratio of the plant is increased due to shoots being more sensitive than the roots to growth inhibition by low water potential (Wu and Cosgrove 2000). Xylem carries a signaling cascade from the roots to the shoots causing some physiological changes ultimately determining the level of adaptation to the stress (Anjum et al. 2011). Abscisic acid (ABA), cytokinins, ethylene, malate, and other unidentified factors have been playing important roles in the root-to-shoot signaling. This long-distance signaling from root to shoot through the transpiration results in stomatal closure, which is a vital adaptation to limited lack of water in the surrounding environment. Stomatal closure happens under loss of turgor pressure due to ABA promoting the efflux of K^+ ions from guard cells (Anjum et al. 2011). ABA is produced in roots as well as in leaves, and levels in both plant parts can increase up to 50-fold due to loss of cell turgor pressure under lack of water or water stress (Guerrero and Mullet 1986). The plant hormone, ABA, has an important role in plant growth and development (Brunner et al. 2015) that it promotes root meristem maintenance and root growth under non-stressed conditions (Sharp et al. 2000), inhibits growth with enhanced ABA concentration under water stress conditions (Nakashima and Yamaguchi-Shinozaki 2013). ABA shows a major signal role besides cytokinins in terms of controlling growth and transpiration (Anjum et al. 2011).

2.6.2.2. Photosynthesis

Photosynthesis (A) is negatively affected by environmental stressors especially water stress. Stress factors have a direct impact on the photosynthetic apparatus. The stressors interrupt all major components of photosynthesis including the thylakoid electron transport, the carbon reduction cycle and stomatal control of the CO_2 supply, water balance and an increased carbohydrate accumulation (Allen and Ort 2001). The ability of the plant to acclimate to diverse environments is directly or indirectly related to their capacity to acclimate at the level of photosynthesis. The plant growth and development are negatively affected by the turn effect of biochemical and physiological process under water stress (Chandra 2003). Water stress brutally impedes the gas exchange parameters of plants and this could be due to a reduction in leaf expansion, diminished photosynthetic machinery, premature leaf senescence, oxidation of chloroplast lipids and changes in the structure of pigments and proteins (Menconi et al. 1995).

2.6.2.3. Stomatal activities

Stomatal and non-stomatal mechanisms are one of the main factors on decreased photosynthetic activity under water stress (Samarah et al. 2009). Stomatal closure is one of the primary responses to water stress which results in declined photosynthesis rate due to water loss and CO_2 absorbance from stomata (Anjum et al. 2011). Anjum et al. (2011) indicated that stomatal closure restricts the leaves of CO_2 and stomata close increasingly with increased water stress. It is well known that the stomatal conductance interacts with the leaf water status. In addition, stomatal closure has an effect on changes in chlorophyll synthesis, functional and structural changes in chloroplasts, and disturbance in the process of accumulation and their transport (Anjum et al. 2011).

2.6.2.4. Chlorophyll content

Chlorophyll is one of the key chloroplast components for photosynthesis, and the chlorophyll content is decreased under water stress due to the symptoms of oxidative stress (Anjum et al. 2011). Chlorophyll content is clearly linked with the photosynthetic rate, which increases biomass production (Ahmadizadeh et al. 2011). Photosynthetic pigments are crucial to plants for absorbing light and production. Water stress caused a large decline and unchanged chlorophyll contents (chl a, chl b and total chl) have been reported in many species, depending on the severity and the duration of water stress (Kulaç et al. 2012; Zhang and Kirkham 1996). Declined chlorophyll content is considered a major trigger of inactivation of photosynthesis (Anjum et al. 2011). The photosynthetic potential is limited with a low concentration of photosynthetic pigments. Mesophyll cells and also bundle sheath cells play a major role in chlorophyll loss in plant response to water deficits due to the fact that photosynthesis occurs in there (Anjum et al. 2011).

2.6.2.5. Tissue water relations

The tissue water relations are so important due to the significance of plant physiology under water stress and it needs to be understood well. Water potential (Ψw) is the straightforward indicator that shows the water availability within the plant throughout soil-plant-air continuum under water stress. Water potential has two primary components (Kozlowski and Pallardy 2002):

$$\Psi w = \Psi s + \Psi p (1)$$

Where Ψs is the solute or osmotic potential and Ψp is the turgor or pressure potential. Pure water is zero in terms of osmotic potential and thus solute potential can be zero or negative. However, turgor pressure increases as water enter a cell that plants can maintain turgor and keep its rigidity.

Water is one of the most important components for plants in terms of the influence of plant water relation characteristics, such as, relative water content (RWC), leaf and stem water potential (SWP), stomatal resistance, the rate of transpiration and leaf temperature. A measure of plant water status is called relative water content, which is higher in the early stage of plant development and declines in later stages of growth, especially when the dry matter accumulates in plants (Anjum et al. 2011). Both water uptake from the roots and water loss by transpiration is related to RWC. When plants are exposed to water stress, the RWC and transpiration rate is decreased (Siddique et al. 2001). The severity and duration of water stress and species has an effect on RWC (Yang et al. 2010). The lack of availability of water and opening and closing stomata affects the plant water relations more than anything else. Also, leaf temperature may be an important factor in controlling plant water status under water stress. Some species maintain water use efficiency by declining water loss, these are called drought tolerant species.

2.6.2.6. Osmolyte accumulation and adjustment

The degree of osmotic adjustment of drought differs from one species to another one and genotypes of plants (Kozlowski and Pallardy 2002). To maintain cell turgor, plants accumulate different types of solutes in the cytosol to reduce osmotic potential (Rhodes and Samaras 1994). Under water stress, the osmotic adjustment not only maintains leaf turgor but also improves water uptake from drying soil with the accumulation of proline, soluble carbohydrates, sucrose, and other solutes (Anjum et al. 2011). All of these accumulations depend on the rate of water stress. Proline accumulation is very important in the stress tolerance. Under water deficits, stress proline is the first osmolyte to be produced in order to reduce injury to cells (Anjum et al. 2011). Anjum et al. (2011) indicated that proline content reached a peak after 10 days of stress, and then decreased under severe water stress. Beside these, proline can act as a signaling molecule to change some cell functions, such as to modulate mitochondrial functions, trigger specific gene expressions, which can be necessary for plant recovery from stress (Szabados and Savouré 2009).

2.6.2.7. Abscisic acid (ABA)

Abscisic acid (ABA) is one of the most important plant hormones for signaling drought or water stress to associate with stomatal closure (Wilkinson and Davies 2002) besides ethylene, cytokinins, amino acids, ROS, sugars, anions and cations, and xylem pH. ABA controls stomatal apertures and is synthesized in roots and leaf mesophyll cells (Kozlowski and Pallardy 2002). Terrestrial higher plants have two guard cells that form a pore in their leaves, and the regulation of gas exchange between atmosphere and plant occurs via pores. Stomatal responses in the leaves involve root-to-shoot signaling via ABA under water stress (Dodd 2005).

When plants are exposed to water stress, the concentration of ABA in plants increase, which triggers an efflux of ions from the guard cells that causes closure of stomata (Mori and Murata 2011). The closure of stomata inhibits gas exchanges via stomata that net photosynthesis and carbon fixation decrease while the plants conserve water (Chaves and Chaves 1991). Thus, ABA plays a crucial role in determining the balance between water loss and productivity or carbon fixation.

2.7. ASSESMENT METHODS FOR WATER STRESS IN TREES

Assessment methods can separate into two sections. First plant-based assessments, and soil-based assessments.

2.7.1. Plant-based Assessments

Plant-based assessments are based on direct and/or indirect measurements of plant water stress such as, stem water potential, photosynthesis, chlorophyll content (Chl a, Chl b and total Chl (a+b), carbohydrate content, and proline content in needles. These measurements can be used to develop better irrigation and fertilization scheduling for plants.

2.7.1.1. Stem water potential

Throughout more than a century, the cohesion tension theory has been used to explain how water moves up to top of all tall plants (Dixon and Joly 1894). This theory explains that, due to negative pressure (tension) on the xylem water column, water moves through stomata, and plants pull the water from soil to upper part of the tree through their roots using the cohesive forces of water. Scholander et al. (1965) approved the use of a pressure chamber for this experiment, and it’s now well known that a pressure chamber can be used to measure the xylem tensile status on cutting branches. The 5-10 cm cutting is placed in the pressure chamber, sealed and pressurized with nitrogen gas gradually until the xylem sap is noticed at the cut surface (Fig. 2.7). To see the sap clearly, a magnifying glass is used.

When the chamber is pressurized with the nitrogen gas, it causes sap flow to reach equilibrium and is known as the balancing pressure. The balancing pressure equals the amount of tension of the vascular sap before the removal of the cutting. To get accurate results the cutting of branches must be prepared carefully and promptly. The foremost challenge with this method is the temperature fluctuations which affect tests. For instance, 0.01 °C change in temperature corresponds to a change in water potential almost to 0.1 Mpa, so to get the most accurate results, this method must be used under stable temperature conditions (Boyer 1995). High transpiration in the leaves when the pressure is released is another factor that affects measurements (Passioura 1991). Although it is a sensitive measurement method, it is very easy and valid method to decide water stress in plants or trees. For example, Wei et al. (2001) discussed all proper techniques, includes pressure bomb, and pressure chamber measurement. Cochard et al. (2001) also contributes this validation, changes in stem water potential measurement by the pressure chamber was correlated with changes in stem water content. This relatively useful, quick method helps to estimate the water potential of tissues through usage of the pressure chamber.

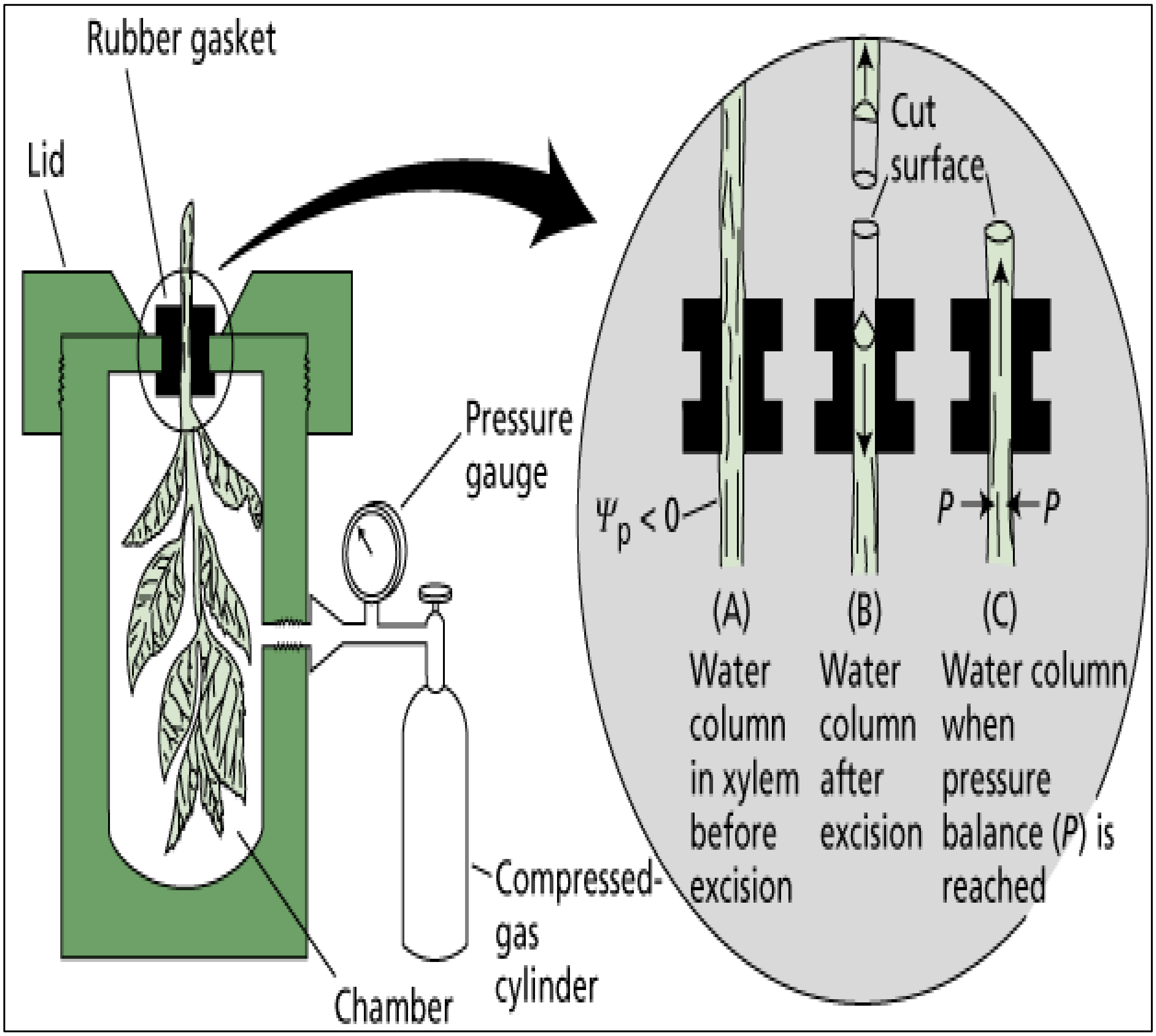

Figure 2.7. Diagram of Scholander pressure chamber. Obtained from http://5e.plantphys.net/.

2.7.1.2. Gas exchange parameters

For more than three decades, a portable photosynthesis measurement system was developed by LI-COR (Lincoln, NE). When they first developed the first portable photosynthesis systems, they also manufactured an infrared gas analyzer that no other company had ever built before. This system gave scientists a chance to work with high performance, reliable, accurate and low-cost field measuring equipment for photosynthesis. Recently, the LI-6400 portable photosynthesis system was developed, and can now provide scientists with a reliable and robust instrument that we often see published in scientific journals (LI-COR 2015) (Fig. 2.8).

Figure 2.8. Picture of LI-COR6400XT and conifer chamber (LI-COR).

Photosynthesis has often been used to explain how water stress affects trees. In recent years, the instrument, LI-COR (LI6400XT, Lincoln, NE), has been used for photosynthesis measurement due its ability to record a variety of measurement, ease of use in the field (portable), and its capacity to take quick snapshots of photosynthetic rate and create a multi-factor response curve. It also provides measurements to examine biochemical restrictions under photosynthetic responses to environmental variables such as light, temperature, CO_2, and humidity.

The photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (TR), and plant water use efficiency (*WUE*) can be measured using a LI-COR (LI6400XT). The LI-COR portable system needs to warm-up and a checklist of things should be done prior to making measurements. After the warm-up, the calibration to a reference CO_2 is needed to maintain at 400 µmol, and air flow rate is held at 500 µmol s^{-1} (LI-COR 2015). To obtain more accurate readings, less air flow rate sets (e.g. 200 µmol s^{-1}) when measuring stressed trees.

2.7.1.3. Chlorophyll pigments

The chlorophyll pigments (Chl a, chl b, and total chl) and carotenoids provide important information about tree physiology (Gitelson et al. 2003). The chlorophylls convert light energy to chemical energy which is stored by leaves and is a function of photosynthesis pigment content, so Chl content is one of the main productions that allows us to find out photosynthetic potential (Curran et al. 1990). Additionally, leaf chl content is strongly linked to plant stress (Merzlyak et al. 1999). Carotenoids have a unique role in photosynthetic membranes, light harvesting, and energy transfer (Young and Brittom 1990) which play an important role for tree physiology (Gitelson et al. 2003). To diagnose the plant physiological status, changes of leaf carotenoids content and their proportion to Chl are widely used; especially during development and when determining acclimation and adaptation to stresses in different environments (Demmig-Adams et al. 1996; Gitelson et al. 2003).

Leaf extraction and spectrophotometric determination for pigment analysis (Arnon 1949) with fresh leaves is one of the best-established new methods in recent years (Gitelson et al. 2003). For the leaf extraction and spectrophotometric determination analysis, fresh needles are needed.

The fresh needle sample is homogenized with a mortar and a pestle in acetone, then centrifuged and measured with a spectrophotometer for chl a, chl b, and carotenoids. This method is very useful, quick, and reliable to help to estimate the leaf chl content, which provides valuable information about a plant's physiological status.

2.7.1.4. Carbohydrate content

Foliar carbohydrate contents change during the year; however, the accumulation of metabolites is led by stress, especially water stress. These metabolites such as amino acids and carbohydrates function as a result of solute accumulation to regulate the intercellular water activity. The solute also works as a water structure regulator (McKersie and Leshen 1994). Under freezing temperature, dehydration may occur, and sugar's function is to protect membranes and proteins (Guy 1990). If a plant reserves carbohydrates, it is at an advantage for when a drought may occur (Virgona and Barlow 1991). The fact that the metabolism consists of carbohydrate or sugar components may be a good evidence that explains its role in the adaptation process (Kerepesi et al. 1998).

For more than 50 years, the phenol sulfuric acid metod described by Dubois et al. (1956) has been the most commonly used method to measure total carbohydrate in needles, because it provides such reliable results. To measure total carbohydrate content, a Hewlett Packard 8452A Photodiode Array UV/Vis Spectrophometer was used; it was set at 490 nm.

2.7.1.5. Proline content

The accumulation of some organic solutes like sugar and proline, lead by metabolic adjustment, is linked to stress adaptation (Yancey et al. 1982). According to Mohammadkhani and Heidari (2008), the accumulation of amino acids, sugars and proline is a common response to abiotic stress. This accumulation effects the osmotic adjustment to the proline content. Proline is needed for osmolytes, and acts as a compatible solute, and accumulates in the cytoplasm (Yancey et al. 1982; Samaras et al. 1995). There is not enough evidence to show that proline is a function of drought induced accumulation (Samaras et al. 1995), although proline is similar to other compatible osmolytes in plants suffering from a lack of water or drought stress. The accumulation of proline is also be part of adaptive responses to drought, which is being influenced by stress (Maggio et al. 2002).

Although there are some procedures to determine the proline content, the procedures described by Bates et al. (1973) have been the most commonly used in recent research. To determine proline content, fresh needles are needed. Although the procedure takes a little while, this method is very reliable and accurately determines proline content using a Hewlett Packard 8452A Photodiode Array UV/Vis-Spectrophometer.

2.7.2. Soil-based Assessments

All plant growth and survival depend on soil, soil moisture and temperature. Water holding capacity of soils can affect the soil moisture and temperature. Although there are several methods of measuring soil moisture and temperature, using a Field Scout TDR 300 Soil Moisture Probe (Spectrum Tech Inc, Plainfield, IL) and using thermocouples (TC-OMEGA®, USA/Canada) for soil moisture and soil temperature, respectively, is one of the best methods for measuring these conditions. These methods are not directly related to drought stress (Baker and Allmaras 1990); however, they are crucial for understanding how irrigation treatments manipulate the soil moisture and temperature. They are easy, quick, and accurate method to assess soil moisture and temperature status (Baker and Allmaras 1990).

2.8. WATER STRESS AND NUTRIENT UPTAKE

Water plays a crucial role during periods of drought, and a plant will resist drought better if it has more available water in the soil (Lange et al. 2002). The capacity of plant roots to uptake water and nutrients via water reduces in plants under water stress, probably because of a decline in nutrient element demand (Alam 1999). Plant metabolism is regulated by essential plant nutrients even under water stress by acting as enzyme activators or cofactors (Nicholas 1975). Water and nutrient availability are a phenomenon that occurs continuously in many different natural environments, with respect to some environmental parameters (Akinci and Lösel 2012). Soils play major roles in plant growth and development. The nutrient uptake of plants is critically influenced by water availability in the soil, root morphology, soil properties, quality and quantity of fertilizers, and the amount of irrigation (Alam 1999). The root structures (e.g. root extension rate, root length, root hair density) plays a huge role in nutrient uptake via water by plants (Akinci and Lösel 2012).

The effect of water stress on mineral uptake and accumulation in plant organs is tough to identify due to different soil types, plant species, and genotypes. However, most studies have pointed out that mineral uptake can reduce with an increase in water stress (Tanguilig et al. 1987; Kirnak et al. 2003; Singh and Singh 2006). Water stress mostly increases uptake of nitrogen, K^+, Ca^{2+}, Mg^{2+}, Na^+, and Cl^- but reduces uptake of phosphorus and iron (Viets 1972). Although, some scientist reported that nutrient uptake is reduced with water stress (Levitt 1980), for example, of phosphorus, K^+, Ca^{2+}, and Mg^{2+} in some plants (Abdalla and El-Khoshiban 2007; Bie et al. 2004). Akinci and Lösel (2012) pointed out that roots lose their ability to function when they get old, and active root tips absorb more nutrients elements in dry soils. Most of the studies stated that nutrient uptake elements are restricted by water stress in plants. Severe water stress can destroy or damage active transport systems, while the presence of numerous ions reacted differently in growth environments (Akinci and Lösel 2012).

2.9. IRRIGATION AND FERTILIZATION INTERACTION

The effects of irrigation and fertilization on plant growth, development, and productivity have been widely studied, but there is not enough research done on the interaction between the two. Recently, there has been an increased interest in investigating the combined effects of fertilization and irrigation on tree physiology and morphology especially in Europe and North America (Nzokou and Cregg 2010a). For example, the study on Loblolly pine (*Pinus taeda* L.) showed that the combination of irrigation and fertilization resulted in greater nutrient uptake efficiency than only fertilization (Albaugh et al. 2008). The combination of irrigation and fertilization had 58% increased biomass compared to control plots (Trichet et al. 2008). Increasing the fertilization level can raise the shoot and root growth, which increases resistance to water stress (Grossnickle 2000). Loblolly pine only responded to fertilization, while there were not any effects on leaf physiology (Samuelson et al. 2008).

The investigations done on fertilization and irrigation mostly focuses on tree performance in field plantations and not on containerized seedlings. The Christmas tree production in containers has been an increasing trend that is becoming more popular than bare rooted production. The conifer tree physiology and morphology are related to irrigation and fertilization, and their combined effects need to be explored and analyzed. Understanding these interactions will provide growers and farmers a better management plan and will reduce growing and production costs.

2.10. WATER-USE EFFICIENCY AND CARBON ISOTOPE DISCRIMINATION

In the Earth's atmosphere, CO_2 is composed of three different carbon isotopes. The majority of CO_2 in the atmosphere has ^{12}C while almost 1 % of the CO_2 has ^{13}C and even smaller amount of is the radioactive species ^{14}C. The genotypic variation in carbon-isotope discrimination

(Δ) have been observed in many plants. The carbon-isotope composition in trees differ mainly due to two stages: (a) the fractionation during diffusion of CO_2 between atmosphere and the intracellular space, and (b) the biochemical fractionation of Rubisco (enzymatic processes) (Ribulose-1,5-biphosphate carboxylase/oxygenase) (Farquhar et al. 1989).

The higher stomatal conductance inhibits the activity of Rubisco due to less $^{13}CO_2$. This takes places in the photosynthates, which in turn reduces the photosynthetic activities, resulting in less plant biomass (Lambers et al. 2008). For plants, $^{13}CO_2$ is heavier than $^{12}CO_2$ diffusing slower than the lighter isotope of $^{12}CO_2$, and the discrimination occurs against the heavy isotope due to the biochemical properties of Rubisco (Lambers et al. 2008). The correlation between isotopic carbon ration (δ13C) values and both the intracellular CO_2 concentration (C_i) and photosynthetic *WUE* has been observed in many studies (Lambers et al. 2008; Cregg 2004). Carbon isotopes discrimination (Δ) or isotopic carbon ratio (δ13C) has been compared with gas exchange measurements and water use efficiency, which is used for analyzing drought tolerance in plants (Cregg 2004). Discrimination against ^{13}C changes with increased *A* or decreased *gs* due to increasing the ratio of *A/gs*. In gas exchange measurement, the negative correlation between discrimination against ^{13}C and instantaneous *WUE* have been observed in many species, such as Douglas-fir (Zhang et al. 1993), black spruce (Flanagan and Johnsen 1995), western larch (Zhang and Marshall 1994), *Pinus ponderosa* (Zhang and Cregg 1996), Scots pine (Cregg and Zhang 2001), and four conifer species (*Picea glauca* var. *densata, Picea pungens, Abies fraseri*, and *Pinus strobus*) (Taylor et al. 2013).

With a determination of drought tolerance in plants, the carbon isotope discrimination provides some advantages based on instantaneous *WUE* (Farquhar et al. 1989). Carbon isotope discrimination provides some information about the relationship of *A/gs* in formed tissue sample over the period while *A/gs* may not deliver decent information of overall *WUE* during the gas exchange measured of intrinsic *WUE* (Cregg 2004). On the other hand, the isotope discrimination on samplings such as on annual rings from increment cores (Macfarlane et al. 1999), climatic conditions, and weather patterns provide an overall *WUE* over the years (Cregg 2004).

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CHAPTER THREE

DO VARIOUS CONIFERS RESPOND DIFFERENTLY TO WATER STESS? A COMPARATIVE STUDY OF WHITE PINE, CONCOLOR FIR AND BALSAM FIR

To be submitted to New Forest.

ABSTRACT

Two-year-old (plug+1) containerized Balsam fir [*Abies balsamea* (L) Mill.] and Concolor fir [*Abies concolor*], and one-year-old bare-root Eastern white pine [*Pinus strobus* (L.)] transplants were grown under variable watering in a factorial greenhouse experiment with the goal of identifying plant morphological and physiological traits of these species' in response to irrigation application. Relative root collar diameter (RRCD), relative height growth (RHG), and relative root length (RRL) were measured as growth parameters. Stem water potential (Ψ), net photosynthesis (*A*), and stomatal conductance (*gs*) were measured twice (June 15 – August 15, 2015). Water use efficiency (*WUE= A/gs*) was determined as the ratio between *A* and *gs*. Foliar potassium (K^+) and calcium (Ca^{+2}) concentrations were determined by atomic absorption spectroscopic methods. For fir species, well-watered seedlings had significantly higher RRCD, RHG, and RRL than medium and low irrigated seedlings. Balsam fir and white pine seedlings had a higher stem water potential (Ψ) than concolor fir transplants under severe stress. Fir species also had higher *A*, *gs*, and a lower *WUE* than white pine seedlings. White pine seedlings had a lower foliar potassium (K^+) concentration compared to the two fir species, and balsam fir had the highest foliar calcium (Ca^{+2}) concentration. Balsam fir grew more and had higher gas exchanges compared to white pine under water stress due to their ability to maintain higher water uptake despite a reduced water content in the soil. However, white pine had higher drought tolerance compared to fir species because of the ability to withstand water stress through the mechanism of reduced photosynthetic activities and growth, to minimizing water loss, and increase water uptake.

Key words: Water stress, irrigation, morphology, physiology, drought-tolerant.

3.1. INTRODUCTION

Water stress is key abiotic stressor limiting plant growth and productivity among the environmental stresses (Shao et al. 2009). The frequency and intensity of plant water deficits are expected to increase due to the ongoing escalation in atmospheric CO_2 concentrations and linked warmer temperatures in temperate forest (Meehl et al. 2007). Pine and fir species have sufficient precipitation to maintain soil moisture in their natural habitat supporting both tree growth and survival (Gilliam 2016) but the future is uncertain.

These species are commercially grown in different environmental settings, however, which may increase challenges such as low water accessibility and increased temperatures. Under these conditions, plants respond differently to various environmental stresses (Bhattacharjee and Saha 2014). Species have varying abilities to acclimate to and to survive under disparate hydraulic and water conditions (Mcdowell et al. 2008; Allen et al. 2010).

Water stresses trigger a variety of plant responses, ranging from plant growth and productivity to altered gene expression and cellular metabolism (Shao et al. 2008a). Numerous physiological and chemical process are involved in responses to water stress in plants. Many biological macro- and micro- molecules, such as nucleic acids, proteins, carbohydrates, lipids, hormones [Abscisic acid (ABA), indole-acetic acid (IAA)], ions [potassium (K^+), Calcium (Ca^{+2})], free radical, and mineral elements are involved in the complex physical-chemical process of drought tolerance (Shao et al. 2008b). For instance; K^+ and Ca^{+2} play a crucial role in stomatal opening and closure. Increased K concentration causes stomatal opening while increased Ca^{+2} causes stomatal closure (Thiec et al. 1995). Several factors affect plant responses to water stress. These include the plant's growth stage, cultivar genetics, and the intensity and duration of stress (Ahmadizadeh et al. 2011).

Under water stress, plants have the capacity to develop morphological and physiological adaptive traits to alleviate water stress effects and to insure survival and growth (Chaves et al. 2002; Kozlowski and Pallardy 2002). This is mostly dependent on differences in hydraulic characteristics and patterns of water use (Allen et al. 2010). Xylem water potential changes due to variations in plants stem water storage caused by increased transpiration and xylem water flux (Meinzer et al. 2009). To reduce water loss by transpiration, strong stomatal control is needed to minimize stem water potential changes as soil water content is reduced. However, this in turn would restrict plant carbon gain in photosynthesis and CO_2 intake (Brodribb et al. 2007). Moreover, plants possess mechanisms such as signal transduction in guard cells to control stomatal aperture. For instance, in response to water stress, plants synthesize abscisic acid triggering the closing of stomatal pores and inhibiting stomatal opening (Mäser et al. 2003).

Mäser et al. (2003) states that plants with developed network systems in their organs, such as guard cells in leaf epidermises, can signal within a single cell to respond to water stress. In the signaling process of stomatal closure, K^+ in plant tissues not only plays a vital role of physiology of plants (Paoli et al. 2005). It also controls foliar water content (Babita et al. 2010) and indirectly, the stomatal functions (Fernández et al. 2006). Potassium (K^+) is vital for plants on drier sites or during drought conditions for a plant's adaptation to water stress (Cakmak 2005) and increased resistance to drought conditions (Egilla et al. 2005). Calcium impacts physiological functions and plant responses to environmental changes through hormonal signals increasing the ability to adapt to drought conditions (Hepler and Wayne 1985).

Tree species in the same family can respond to environmental stress differently. Previous studies have indicated that Douglas-fir [*Pseudotsuga menziesii*] and lodgepole pine [*Pinus contorta*] differ in terms of physiological characteristics and drought tolerance (Martinez-Vitola et al. 2004). It is stated that lodgepole pine has a greater tolerance to drought stress, while Douglas-fir is better able to prevent the loss of xylem hydraulic conductivity (Piñol and Sala 2000). This indicates that other physiological and structural features of lodgepole pine may compensate to avoid shoot and stem water potentials from reaching levels that would cause significant loss of hydraulic conductivity under field conditions (Martinez-Vitola et al. 2004).

The adverse effects of climate change on conifer species such as pine and fir species include increasingly facing drought in their natural habitat and plantation settings. It is known that soil water deficiency induces physiological stress and affects tree species differently depending on specific functional traits such as leaf phenology and phenotypic plasticity (Camarero et al. 2015). For example, pine species close stomata to avoid water loss via transpiration and embolism in the xylem, which reduces carbon assimilation (Hubbard et al. 2001). This, in turn, diminishes growth and productivity (Tyree and Sperry 1988). Even closely related pine species such as Scots pine (*Pinus sylvestris* L.) (Martínez-Vilalta and Piñol 2002) and black pine (*Pinus nigra* Arn. subsp. *Salzmannii* (Dunol) Franco) (Sánchez-Salguero et al. 2012, 2015) respond differently to water stress. These studies showed that black pines are better able to withstand drought stress compared to Scots pine with a water stress-avoidance strategy such as rapid stomatal closure and relatively higher soil water content. Also, other possible physiological responses to water deficit include the loss of turgor pressure, reduction of growth and cell enlargement, limitation of gas exchanges (Jaleel et al. 2009), and declining rates of photosynthetic pigments (Yordanov et al. 2000).

Among conifer species, Eastern white pine [*Pinus strobus* (L.)], concolor fir [*Abies concolor* subsp. *concolor* ‘Cibola’] and balsam fir [*Abies balsamea* (L.) Mill ‘Cooks’] are preferred to use for this study due to economic importance, renewable resources, desirable color for ornamental value such as Christmas tree production and landscaping. Eastern white pine is native to eastern North America, fast-growing softwood, and has been widely used for reforestation, landscaping and Christmas tree production (Wendel and Smith 1990). Balsam fir is native to most of eastern and central Canada and the northeast United States (Frank 1990), it is also slow-growing tree, and has been widely used for a Christmas tree due to its strong scent and dark green needles (Cregg 2016). Concolor fir is native to the western United States including Colorado, Arizona, Utah, Idaho, Nevada and New Mexico, and is widely distributed in the southern Rocky Mountains (Laacke 1990). Concolor fir trees receive low rainfall compared to white pine and balsam fir in their native range and are adapted to drier environments. In their natural habitat, white pine receives 510-2030 mm precipitation annually (Wendel and Smith 1990) while balsam fir 'Cooks' and concolor fir 'Cibola' receive 760-1100- and 520-890-mm precipitation, respectively (Frank 1990; Laacke 1990).

It has been suggested that species such as Eastern white pine and concolor fir could be drought-tolerant (Wood, 2006; Wennerberg 2004a, 2004b). However, balsam fir performance in unfavorable environmental conditions is not well established. Understanding how these species respond to low water availability is ever more important due to global climate change. These species are very economically important for various intensive production systems and there are indications that they will continue to be planted in large scale afforestation programs. By improving the knowledge of underlying mechanisms and responses to water stress, managers will better be prepared for changing precipitation patterns and soil water conditions. In this paper, we hypothesized that because of their tendency to produce longer taproots during early growth period, white pine will have a better ability to withstand water stress compared to both concolor and balsam fir. Specific objectives of this study were: (i) to investigate the morphological and physiological responses of species to water stress; and (ii) to determine the differences and/ or similarities between species in terms of response parameters under water stress.

3.2. MATERIALS AND METHODS

3.2.1. Site description

This experiment was conducted in a greenhouse running east to west at the Tree Research Center (TRC) on the Michigan State University campus, East Lansing, Michigan in 2015. The coordinates of the TRC are 42°65’N and 84°42’W. The greenhouse was covered with a double layer of clear plastic. The maximum and minimum temperature were 27.04 and 18.52 °C in the greenhouse throughout experimental period (June 15 - August 15).

3.2.2. Plant material and containerization substrates

Two-year-old (plug+1) containerized Concolor fir [*Abies concolor*] ‘Cibola’ (New Mexico) and Balsam fir [*Abies balsamea* (L.) Mill.] ‘Cooks’ (New York), and one-year-old bare-root Eastern White Pine [*Pinus strobus* (L.)] (Michigan) transplants were obtained from a commercial nursery (Vans Pine nursery, West Olive, MI) on May 13, 2015. Upon reception, tree species were transplanted into black cylindrical 3-gal (11.2 liter) plastic containers with Fafard 52 potting mix (Conrad Fafard, Inc. Agawam, MA). The potting mix contains Canadian sphagnum peat moss (30%), processed pine bark, perlite, vermiculite, wetting agents and dolomitic limestone, with a pH range of 5.5 to 6.5 after wetting. The 48 white pine seedlings were root pruned to 15 cm to prepare the bare roots for planting. The 120 fir seedlings were simply transplanted into containers. Following the transplanting, trees were well-watered to establish them in the containers before the treatments were started on July 15, 2015.

Each container received 60g of granular fertilizer applied as top dress at the beginning of the season. The fertilizer, Osmocote Plus 15-9-12, is a 5-6 month controlled-released formulation containing 15 % total nitrogen (N), 9 % of available phosphate (P_2O_5) (P) and 12 % of soluble potash (K_2O) (K) (Everris NA Inc, Dublin, OH). In addition to these macronutrients, the fertilizer also contains several micronutrients including 1% magnesium (Mg), 2.3% sulfur (S), 0.02% boron (B), 0.05% copper (Cu), 0.45% iron (Fe), 0.06% manganese (Mn), 0.02% molybdenum (Mo), and 0.05% zinc (Zn).

3.2.3. Irrigation treatment

Before water stress started, five containerized seedlings were used to determine plant water requirements according to the following procedure for each species. The five containerized transplants were well-watered and allowed to drain gravitationally for 2 days. At the end of the 2-days, 1000 mL of water was applied to each container and allowed to run off two hours. Then, the initial weight of the container was measured for each container. The containers were allowed to drain for 2 days gravitationally with the drained water being collected. After the two days, the final weights of the containers were measured and recorded again. The difference between the initial and final weights of each containerized plant was assumed to correspond to the weight of the water utilized by the tree plus the evaporation from the container’s substrate. This process was replicated 3 times to determine how much water each test seedling in the container used in a week.

Irrigation treatments were then established at 25, 50, and 100 % of the initial water requirement as determined above. This value was increase by approximately 10 % to meet the increasing water demands later in the growing period. The three irrigation volumes corresponding with each weekly treatment are: 1) low (high stress) (750 ml), medium (mild stress) (1500 ml) and high (Well-watered) (3000 ml) for balsam and concolor fir; 2) low (high stress) (900 ml), medium (mild stress) (1800 ml) and high (Well-watered) (3600 ml) for the pine seedlings. Manual applications of water started from the beginning of the experiment until the end of the experiment.

3.2.4. Morphological responses

Stem height, root length, and root collar diameter growth were measured at the beginning and at the end of the experimental period. A digital caliper was used to measure all trees root collar diameter at the soil surface while a tape measure was used to measure stem height from the soil level to the terminal shoot tip while a tape measurer was used to measure stem height and root length. Initial root length was measured before transplants placed in containers. Relative height growth (RHG), relative root collar diameter growth (RRCD) and relative root length (RRL) were calculated as the difference between the end of season measurement and the initial measurement divided by initial measurement.

3.2.5. Stem water potential

Stem water potential (SWP) was measured using the pressure chamber method with a plant water status console model 3115 (Soil Moisture Equipment Corp, Santa Barbara, CA) according to Turner (1988). Five cuttings were taken from the new year's growth of length ranging from 5-10 cm for each treatment. The samples were placed in the pressure chamber, and the chamber was then pressurized. A measurement was taken when the cut surface became wet or shiny indicating xylem water coming back to the surface. Measurements were taken at midday between 11:30 A.M – 2:30 P.M. Soil moisture content was measured seven times during the experimental period in each pot for each treatment using a Field Scout TDR 300 Soil Moisture Probe (Spectrum Tech Inc, Plainfield, IL).

3.2.6. Photosynthesis

Photosynthesis measurements were conducted on three randomly selected trees from each treatment using a LI-COR (LI6400XT, Lincoln, NE) conifer chamber with a RGB light source (6400-18A). The instrument was matched before each measurement and the reference CO_2 was maintained at 400 µmol, and the air flow rate was held at 500 µmol s^{-1}. Three readings were taken on current years developed branches on each tree between 11:00 AM and 3:30 PM. Measured shoots were cut after each measurement and stored in a cooler at ±5 °C. All gas exchange parameters were expressed on the projected needle area basis. Needles were removed from the shoot, images of all the needles were captured with a flatted scanner, and ImageJ software was used for image analysis (Rashband 2012). The measured needle area for each specimen was entered as section in the LI-COR system for adjustment of gas exchanges parameters previously measured in the greenhouse for the specimen.

Several physiological parameters including the net photosynthesis (*A*) and stomatal conductance (*gs*) were measured in mature needles from current year exposed to full sun using a conifer chamber with a red/green/blue LED light source (Li-6400xt-02, LI-COR) maintaining a quantum flux of 1800 µmol s^{-1}. Water use efficiency (*WUE*) was calculated as $WUE = A/gs$. The ratio of carbon gain to water loss is defined as *WUE* (Sinclair et al. 1984).

3.2.7. Potassium and calcium concentration on needle tissues

Foliar tissue samples were collected for analysis of foliar potassium and calcium concentration. Tissues were obtained from all plants in each plot. Samples were stored at 4 ºC in sealed plastic bags prior to the analysis. Samples were oven dried at 65 ºC (± 5 ºC) for at least 48 hours. Afterwards, samples were ground into a fine powder. Approximately, 0.3 g of powder from each sample were mix with H_2SO_4 (4.5 mL) and H_2O_2 (1.5 mL) in a 100-mL digestion tube to pre-digest overnight. The digestion tubes were gradually heated in a digestion block (AIM600) to 340 ± 10 ºC (increasing 5 ºC every hour until 340 ºC) where they stayed at a constant temperature for an hour. After the digestion was completed, samples were diluted with distilled water and placed on a centrifuge to mix the solution. Potassium and calcium analysis were performed on an aliquot of digested tissue on an atomic absorption spectrometer (Aanalyst 400, Perkin Elmer, Waltham, MA).

3.2.8. Data analysis

The experimental setup was a factorial design with 3 species (White pine, concolor fir, balsam fir), and 3 irrigation levels [Low, Medium, High]. Each treatment had 5 seedlings for the fir species and 4 seedlings for the pine species totaling 168 individual trees. A level of significance of $\alpha=0.05$ was used for inferring any statistical significance. Statistical analyses were performed using SAS software 9.4 (SAS Institute Inc., Cary, NC). All variables were tested for normality using PROC TRANSREG, histogram and box-cox. Data that were not normal were transformed as appropriate, such as RRCD, RHG, and *gs* were normalized using a log transformation when RRL and WUE were normalized using a square root transformation. PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. Effect over species, irrigation level and time were analyzed using repated measures within PROC MIXED. Mean separation using Tukey's adjustment was used to compare with all significant responses. We also determined both simple linear regressions for allometric variables and correlation coefficients for net photosynthetic rates and stomatal conductance in species. In addition, an average linkage, K-means cluster method was used to compute cluster grouping of three conifer species based on *gs* and *A*. K-means cluster analysis was conducted using SYSTAT 13 software (Systat Software Inc., Chicago, Illinois, USA).

3.3. RESULTS

3.3.1. Morphological traits

3.3.1.1. Relative root collar diameter (RRCD), height (RHG) and root length (RRL) growth

Balsam fir seedlings showed the highest RRCD (0.68 mm/mm) followed by concolor fir (0.58 mm/mm) and white pine (0.27 mm/mm) (Table 3.1). Irrigation treatment was significant

(P<0.05), and well-watered seedlings had higher RRCD compared to medium and low irrigated transplants. There was no significant effect on the interaction of species and irrigation (SxI) (P>0.05) (Table 3.2).

Balsam fir seedlings had the highest RHG (0.36 cm/cm) followed by concolor fir (0.30 cm/cm), and white pine (0.21 cm/cm) (Table 3.1). Stressed seedlings had lower RHG values (0.28 and 0.29 cm/cm, respectively) while well-watered seedlings showed higher values (0.30cm/cm) (Table 3.1). Under the interaction of SxI treatments, increased irrigation only increased the RHG for balsam fir seedlings.

Table 3.1. Mean and standard errors of morphological traits under three species and irrigation combination.

RRCD (mm/mm)	Species			
Irrigation	Balsam fir	Concolor fir	White pine	Mean
Low	0.61±0.02 b	0.51±0.02 b	0.17±0.02 c	0.43±0.01*C
Medium	0.66±0.02 b	0.53±0.02 b	0.28±0.02 b	0.49±0.01*B
Well-watered	0.78±0.02 a	0.70±0.02 a	0.36±0.02 a	0.61±0.01*A
Mean	0.68±0.01*A	0.58±0.01*B	0.27±0.01*C	

RHG (cm/cm)	Species			
Irrigation	Balsam	Concolor	White Pine	Mean
Low	0.33±0.01 b	0.31±0.01 a	0.20±0.01 a	0.28±0.01 B
Medium	0.37±0.01 ab	0.29±0.01 a	0.21±0.01 a	0.29±0.01 AB
Well-watered	0.39±0.01 a	0.30±0.01 a	0.21±0.01 a	0.30±0.01 A
Mean	0.36±0.01 A	0.30±0.01 B	0.21±0.01 C	

RRL (cm/cm)	Species			
Irrigation	Balsam	Concolor	White Pine	Mean
Low	0.81±0.01 a	0.80±0.01 a	0.73±0.01 b	0.79±0.00*AB
Medium	0.81±0.01 a	0.83±0.01 a	0.76±0.01 a	0.80±0.00*A
Well-watered	0.80±0.01 a	0.76±0.01 b	0.74±0.01 ab	0.77±0.00*B
Mean	0.81±0.00*A	0.81±0.00*A	0.74±0.00*B	

Note: Means within column followed by the same capitalized letter are not significantly different at 0.05 level for species. [b] Means within row followed by the same capitalized letter are not significantly different at 0.05 level for irrigation. [c] Means and standard errors in tables followed by same lowercase letter are not significantly different at 0.05 level for the combination of species and irrigation.

White pine seedlings had the lowest RRL (0.74 cm/cm) compared to concolor and balsam fir (0.80, 0.81 cm/cm respectively) (Table 3.1). There were no significant differences between fir species in terms of RRL.

Table 3.2. Degrees of freedom (df), F values for the repeated measures of analysis of variance for relative root collar diameter (RRCD), relative height growth (RHG), relative root length (RRL) among three species and three irrigation levels.

		F-values		
Source of variation	df	RRCD	RHG	RRL
Between subjects				
Species (S)	2	273.24***	219.81***	98.67***
Irrigation (I)	2	53.59***	3.65*	0.36
Within subjects				
SxI	4	1.44	2.83*	2.30

Note: Significant levels for repeated measures are given as corrected probabilities: * = P < 0.05; ** = P < 0.01; *** = P <0.001.

3.3.2. Physiological parameters

3.3.2.1. Stem water potential

Species (S) and irrigation had a significant effect on Ψ (P<0.05) (Table 3.3). Balsam fir seedlings had statistically higher Ψ when concolor fir had the lowest Ψ when species as the single factor. There were no significant differences between white pine and concolor fir seedlings. Well-watered treatment seedling had higher Ψ and followed by medium and low watered treatment had the lowest values.

Under the interaction of SxI, the well-watered treatments that received high levels of irrigation maintained higher Ψ when compared to medium and low irrigation for each species. White pine and balsam fir seedlings had higher Ψ compared to concolor fir under each irrigation treatment (Fig. 3.1). Balsam fir had higher Ψ compared to white pine and concolor fir under well-watered treatment. However, under low watered treatment, white pine had higher Ψ than balsam

Table 3.3. Degrees of freedom (df), *F* values for the repeated measures of analysis of variance for stem water potential (Ψ), net photosynthesis (*A*), stomatal conductance (*gs*), and water use efficiency (*WUE*) among three species and three irrigation levels.

		F-values			
Source of variation	df	Ψ	*A*	*gs*	*WUE*
Between subjects					
Species (S)	2	4.35*	232.12***	135.10***	6.41**
Irrigation (I)	2	35.55***	4.12*	7.44**	6.22**
Within subjects					
SxI	4	0.69	3.36*	6.40**	8.63***

Note: Significant levels for repeated measures are given as corrected probabilities: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

fir where balsam fir was affected more from water stress, however there were no statistical differences between these two species.

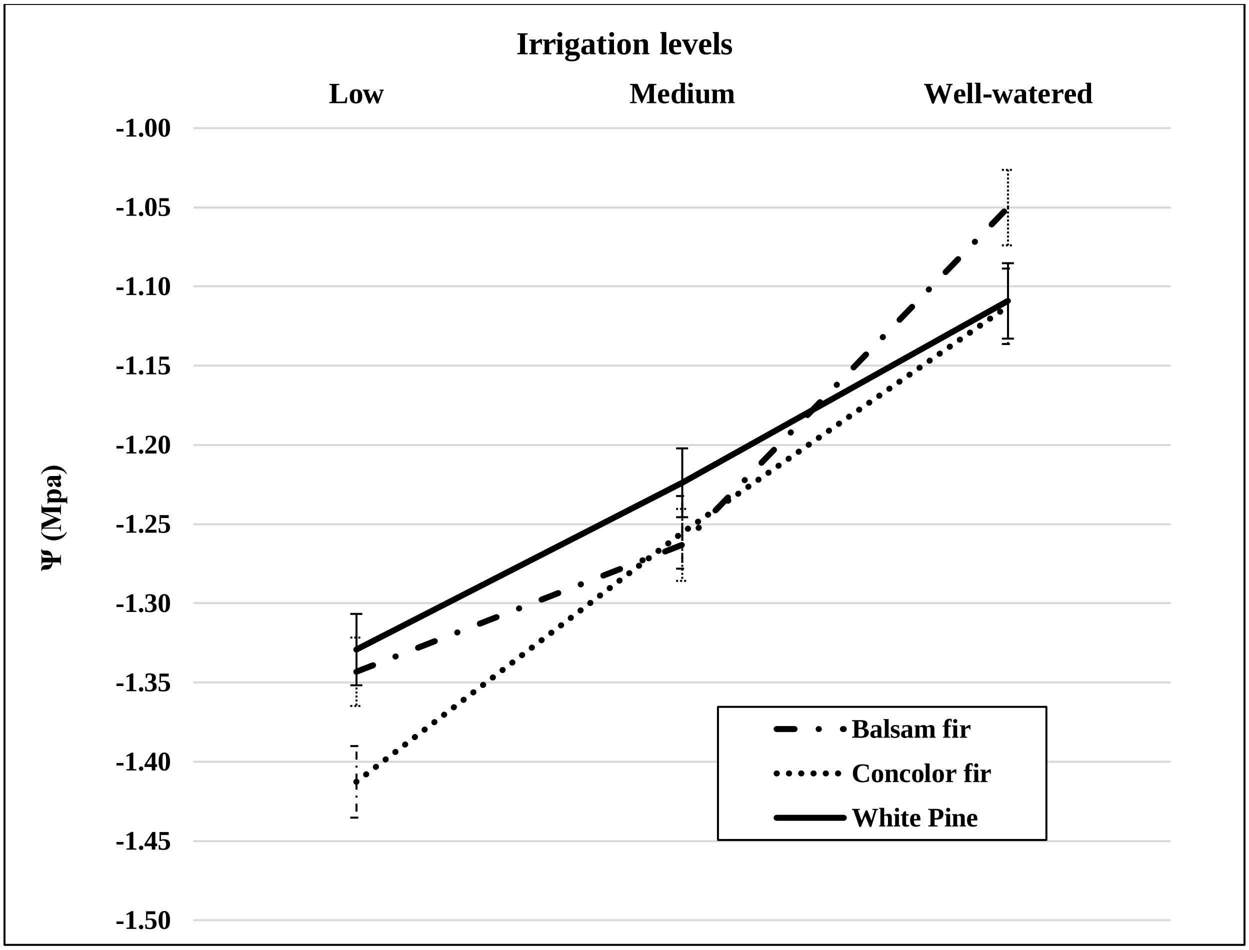

Figure 3.1. Mean and standard errors of *Pinus strobus*, *Abies balsamea* and *Abies concolor* seedlings of midday stem water potential (Ψ) (Mpa) under three irrigation levels.

3.3.2.2. Net photosynthesis (A)

Species and SxI had a significant effect on *A* ($P<0.05$) (Table 3.3). White pine seedlings had the lowest photosynthetic rate when compared to the two fir species. The interaction between SxI was significant ($P=0.012$) when net photosynthesis increased with increasing irrigation (Well-watered > Medium = Low). Low and medium-watered white pine seedlings had the lowest net photosynthesis values compared to all other SxI interactions (Fig. 3.2). Balsam and concolor species seedlings under each combination of S and I had significantly higher net photosynthesis values than white pine seedlings (Fig. 3.2*).*

3.3.2.3. Stomatal conductance (gs)

Species (S), irrigation (I), and SxI was significant for gs ($P<0.05$) (Table 3.3.). White pine seedlings had a lower *gs* (0.51 mol m^{-2} s^{-1}) values when compared to balsam (0.11 mol m^{-2} s^{-1}) and concolor fir (0.10 mol m^{-2} s^{-1}) for stomatal conductance. Stomatal conductance increased with increasing irrigation (Well-watered > Medium = Low) across all treatments. Under the interaction of SxI treatments, two fir species had no statistical differences within that they had higher *gs* values compared to white pine seedlings with all interactions of SxI (Fig. 3.2). Low and medium watered white pine seedlings had the lowest *gs* compared to well-watered white pine seedlings and all combination of SxI for balsam and concolor fir (Fig. 3.2).

The relationship between *A* and *gs* was shown on Fig. 3.3. The significant relationship between *A* and *gs* was highest on white pine ($r^2 = 0.53$) followed by balsam fir ($r^2 = 0.30$) where the lowest relationship on concolor fir ($r^2 = 0.53$). In general, both well-watered seedlings had higher *gs* and *A*, however, increased *gs* more rapidly increased *A* linearly in white pine compared to fir species. Cluster analysis classification technique was used to group low and well-watered treatments with and within each species in terms of *gs* and *A*. Three distinct cluster groups can be

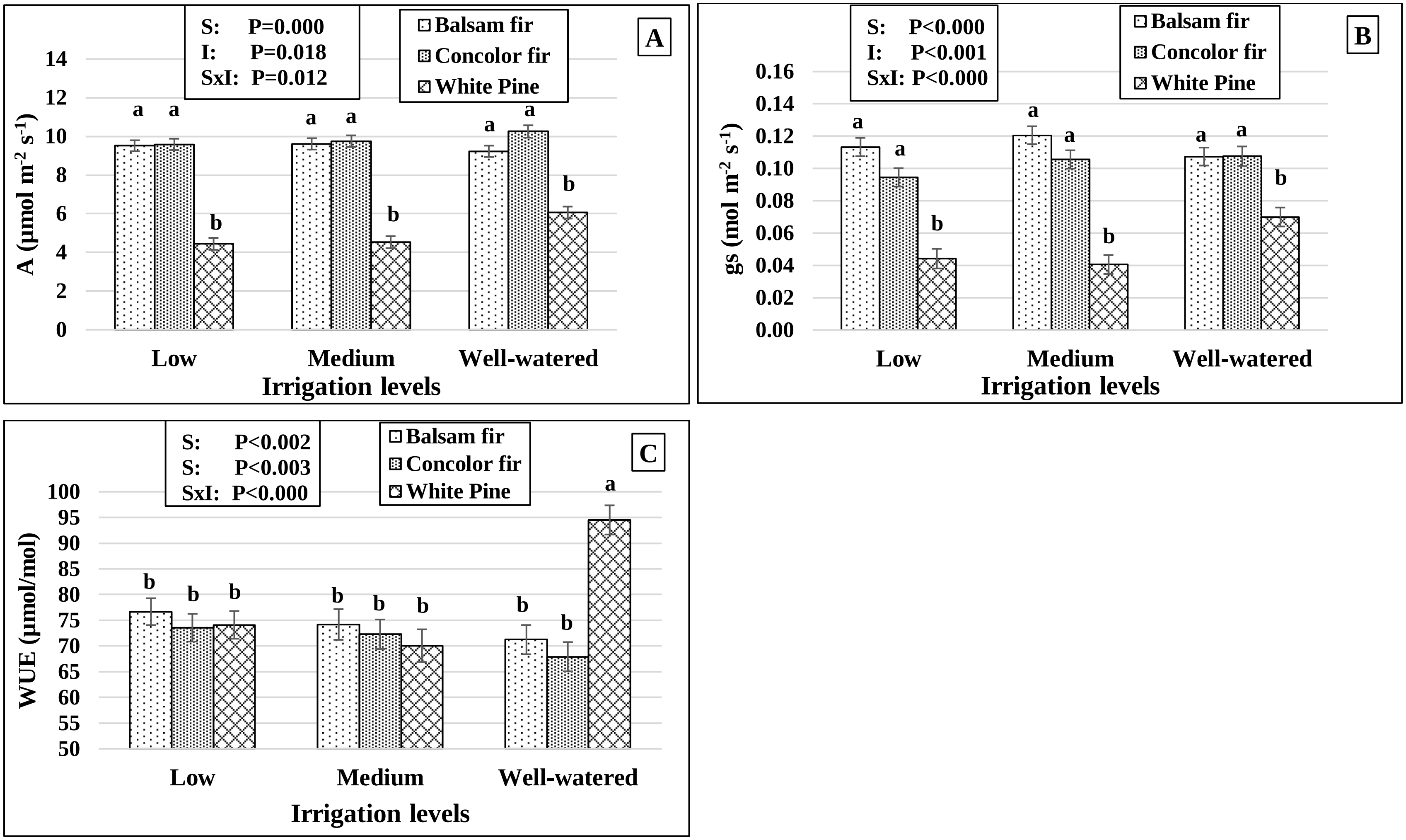

Figure 3.2. Mean, standard errors and p-values of *A*, *gs*, and *WUE = A/gs* under the combination of three species and irrigation levels.

Note: In the figure followed by same letter are not significantly different for within each irrigation treatment (alpha=0.05). *A*: net photosynthesis (µmol CO_2/m^2 needle areas) (Fig. A); *gs*: stomatal conductance (mol H_2O/m^2/needle areas) (Fig. B); *WUE*: water use efficiency (µmol/mol) (Fig. C) among tree species and three irrigation. Balsam fir: *Abies balsamea*, Concolor fir: *Abies concolor*, White pine: *Pinus strobus*.

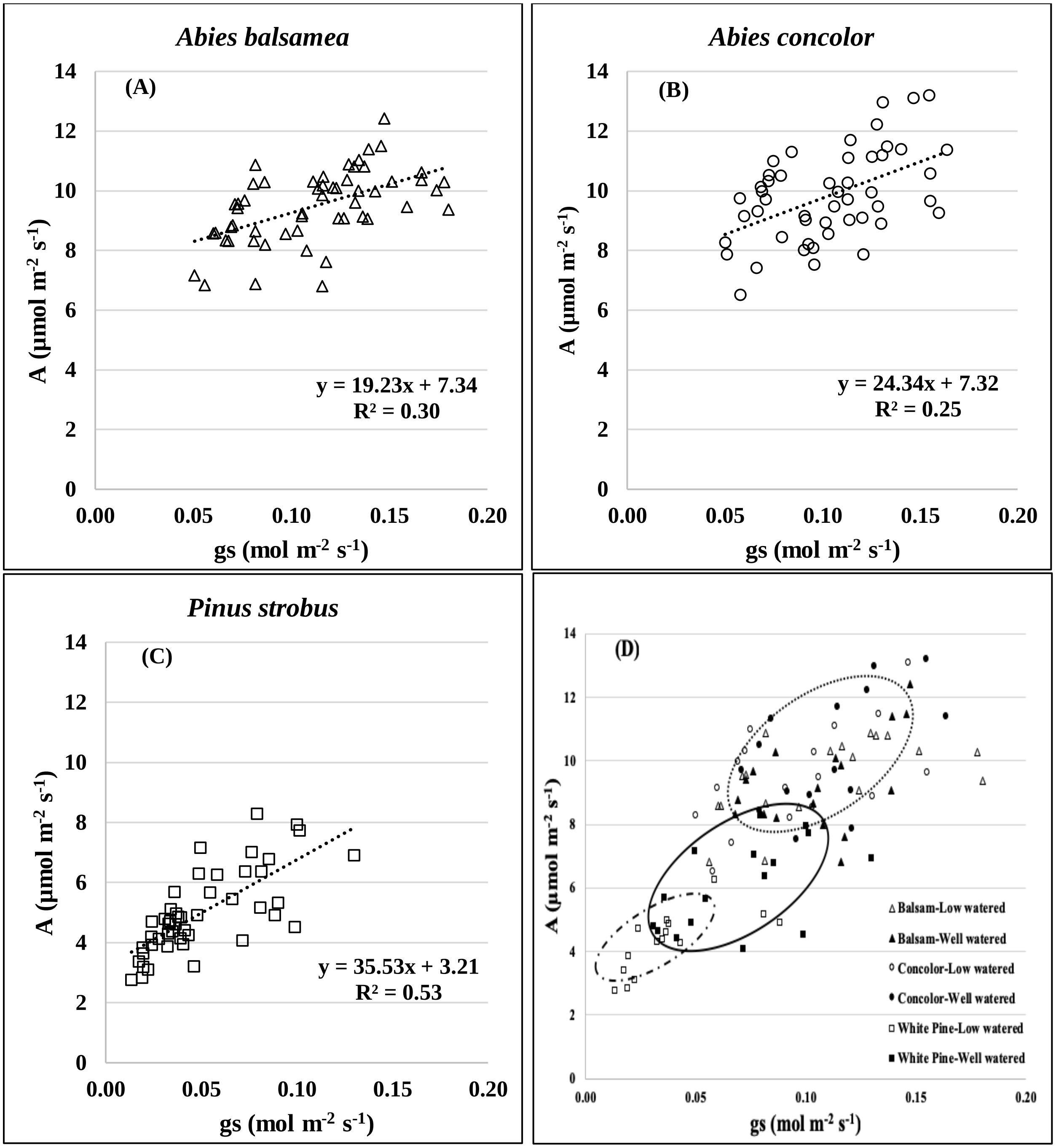

Figure 3.3. Relationship between *A* and *gs* within a) *Abies balsamea*, b) *Abies concolor*, c) *Pinus strobus* species, and d) K-means cluster analysis of the *A* and *gs* for these species under two irrigation levels.

Note: Cluster analysis of A and gs separated species in three groups [White pine low-watered (-.-.-), White pine well-watered (-) and two fir species (…..) under low and well-watered treatments (Fig. 3.3 (D)). *Abies balsamea*: Balsam fir, *Abies concolor*: Concolor fir, *Pinus strobus*: White pine.

identified within species (Fig. 3.3 (D)). Both fir species behaved same without significant changes with irrigation treatments compared to white pine seedlings. However, white pine seedlings clustered within two groups that well-watered seedlings had higher *gs* and *A* values compared to low-watered seedlings.

3.3.2.4. Water use efficiency (WUE)

WUE (%) significantly affected by species (S), irrigation (I), and SxI ($P<0.05$) (Table 3.3). White pine had a higher *WUE* values (79 %) compared to balsam (74 %) and concolor (71 %) and there were no significant differences between two fir species. Well-watered seedlings had higher *WUE* compared to low and medium irrigation. The interaction between SxI was significant ($P<0.0001$) showing that well-watered white pine seedling had higher values (94 %) compared to all other interaction of S and I treatments (Fig. 3.3).

3.3.3. Potassium and calcium concentration on needle tissues

Potassium (K^+) concentration differed among species (S) and irrigation (I) treatments ($P<0.05$). White pine seedlings had lower K^+ concentration (2.36 mg/L) than balsam (2.69 mg/L) and concolor fir (2.94 mg/L) (Fig. 3.4). Moderately stressed seedlings had the highest K^+ concentration (2.87 mg/L) compared mild-stressed (2.81 mg/L) and well-watered seedlings (2.31 mg/L) when examining irrigation as a single factor.

Calcium concentrations (Ca^{+2}) were only measured on day 17 because day 32 samples had Ca^{+2} concentration zero or close to zero values that these values were removed from statistical analysis. Species (S), irrigation (I), and SxI were significant ($P<0.05$). Balsam fir seedlings had the highest Ca^{+2} concentration (2.75 mg/L) followed by white pine seedlings (2.24 mg/L) and concolor fir transplants had the lowest values (1.71 mg/L) when examining species as a single factor.

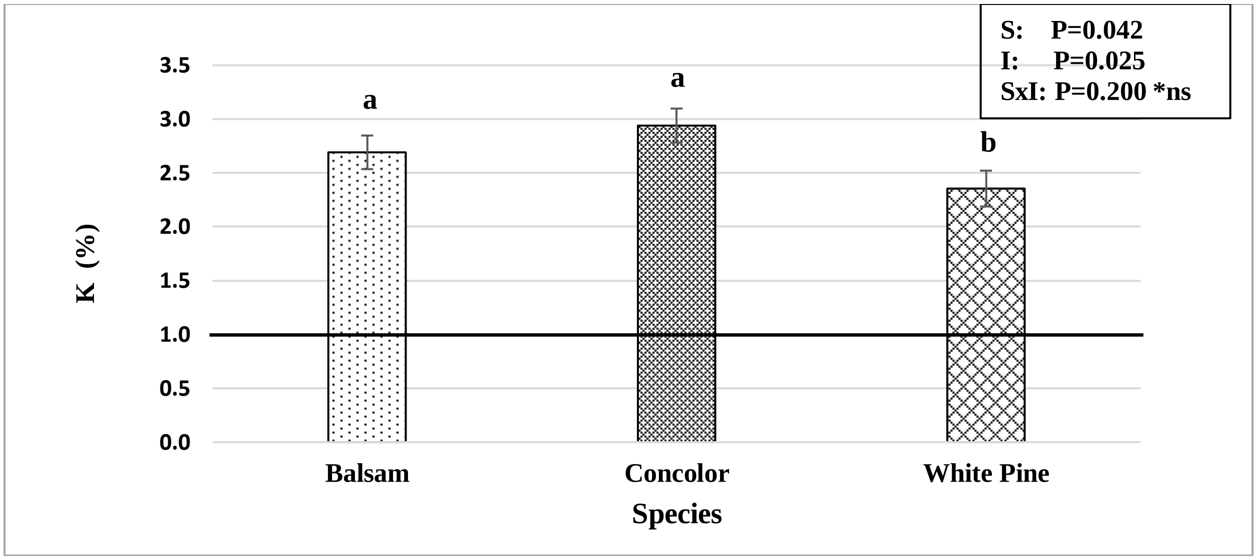

Figure 3.4. Mean and standard errors of *Pinus strobus*, *Abies balsamea* and *Abies concolor* seedlings of needle potassium (K^+) concentration (%).

Note: Means and standard errors in the figure followed by same letter are not significantly different for tree species (alpha=0.05). Sufficient concentration for K in conifer species is approximately 1 % (Cregg 2005) that is shown in this graph with a bold line. Species (S) and irrigation (I) were significant (P<0.05) while SxI combination was not significant (P>0.05).

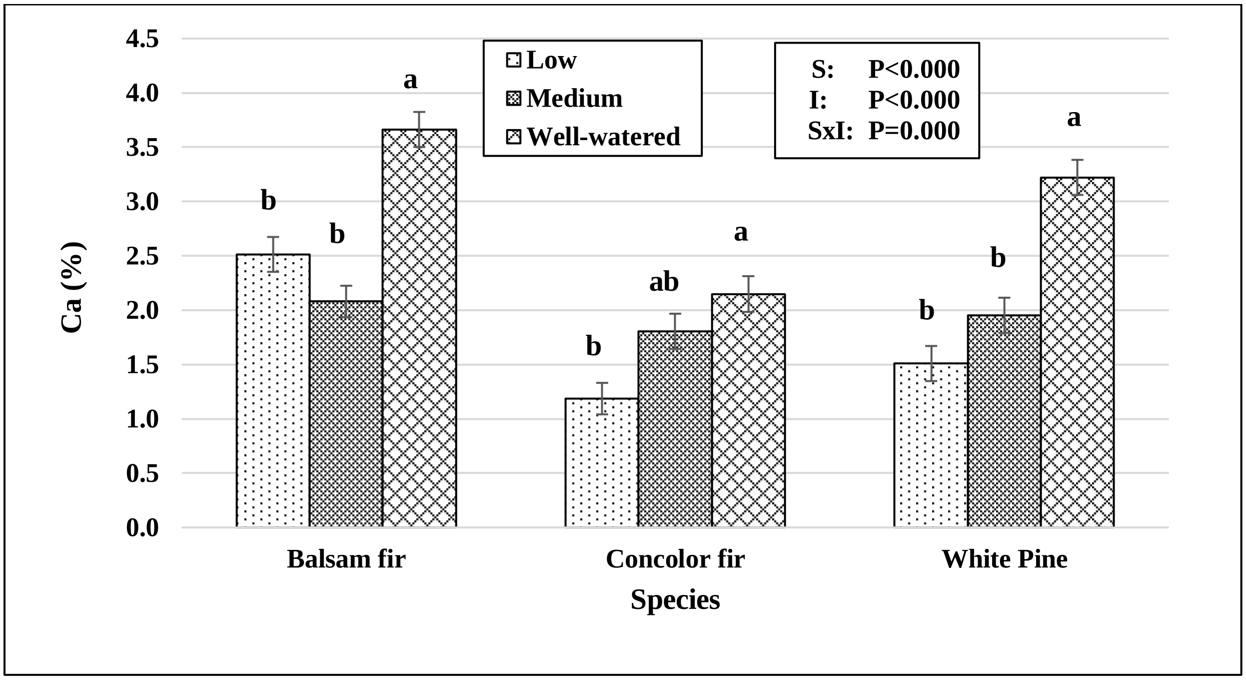

Figure 3.5. Mean and standard errors of *Pinus strobus*, *Abies balsamea* and *Abies concolor* seedlings of needle calcium (Ca^{+2}) concentration (%) under three irrigation regimes.

Note: Means and standard errors in the figure followed by same letter are not significantly different for the irrigation within each species (alpha=0.05).

Well-watered seedlings had the highest Ca^{+2} concentration (3.01 mg/L) compared to medium watered (1.95 mg/L) and low watered (1.74 mg/L) seedlings with no significant difference between medium and low watered treatments. Well-watered balsam fir seedlings had the highest Ca^{+2} concentration (3.66 mg/L) while low watered concolor seedlings had the lowest values (1.19 mg/L) under the interaction between SxI (Fig. 3.5). Increasing irrigation increased Ca^{+2} concentration for concolor and white pine seedlings while increasing irrigation reduced Ca^{+2} concentration (medium) first and then increased Ca^{+2} concentration (well-watered) for balsam fir (Fig. 3.5).

3.4. DISCUSSION

3.4.1. Effect on RRCD, RHG, and RRL response

Overall, species and irrigation significantly affected tree growth and development under water deficiency. Under water stress, pine species tend to increase root growth and/ or relative allocation to roots (Teskey et al. 1987) while fir species tend to increase their height growth (Gower et al. 1992), and our project showed similar results. Balsam fir seedlings had the greatest increase in RRCD and RHG followed by concolor fir and white pine seedlings. While as expected increased irrigation resulted in a subsequent increase in growth parameters, the species did have different response mechanisms to growth under water stress conditions. In general, height growth occurs in early growing season while diameter expansion occurs later in growing season where water availability has a greater impact (Nikiema et al. 2012). Decreasing water availability reduces cell elongation, having a negative effect on both RRCD and RHG while inducing root production. Roots not only may grow deeper into soil to reach available water, but may also act as sensors for shoots regarding water shortage conditions (Hamanishi and Campbell 2011). Increasing root length allows plants to access deeper soil water and is vital for seedlings under water stress (Cregg and Zhang 2001). However, plant stock type selection can influence root growth in drier sites due to different growth capabilities (Grossnickle 2012) that containerized-grown seedlings may have a greater root growth during the first field growing season (Becker et al. 1987; Burdett et al. 1984). In general, containerized seedlings experience less planting stress compared to bare-root seedlings due to stock type differences (Alm 1983; Nilsson and Örlander 1995).

3.4.2. Effect on plant physiology

Stem water potential was used as one of the main indices of water stress in this study. Vapor pressure deficits are elevated in the middle of the day resulting in decreased Ψ in plants (Williams and Araujo 2002). The needle-to-air vapor pressure deficit, needle/plant water status and abscisic acid (ABA) accumulation were physiological factors for stomatal opening and closing (Farquhar and Sharkey 1982). To reduce the negative effects of water deficit, plants tend to close their stomata. However, there are inherent differences between species in terms of water stress responses. This study found that concolor fir seedlings had a lower midday Ψ than the other two species under high water stress while there were no differences between species under medium stress. Another study found similar differences between Douglas-fir and lodgepole pine in terms of patterns of midday Ψ (Andrews et al. 2012). Balsam fir showed the highest Ψ compared to other species when there were no differences between white pine and concolor fir seedlings under well-watered treatment. This shows that species can differ from one another in terms of their response to water stress. Under severe and mild water stress, a more conservative strategy of stomatal conductance regulated by water loss was observed for white pine seedlings in this study. Similar results were observed for Douglas-fir (Domec et al. 2008). However, containerized seedlings may have an improved root growth compared to bare-root seedlings which reduces their resistance to water uptake (Grossnickle and Blake 1987). This may support a greater photosynthesis in containerized seedlings due to a favorable plant water status.

With decreasing water availability, xylem pressure potential declined, and net photosynthetic rate and stomatal conductance decreased in all species. We observed higher *A* and *gs* values in fir species compared to white pine seedlings due to differences in leaf morphology, leaf area measurement technique and the drought-adaptation mechanisms (morphological, physiological, bio-chemical) of tree species. However, the relationship between *gs* and *A* was higher in white pine seedlings compared to fir species seedlings (Fig. 3.3. (A, B, C)). The cluster analysis results were also in line with linear regressions results that fir species seedlings clustered in a group while white pine seedlings separated in two groups. Due to root morphology and length, adaptation mechanisms of species, needle size and shape, fir species had a different cluster than white pine seedlings in terms of *A* and *gs*. Well-watered white pine species had a different cluster that low-watered seedlings while no differences within irrigation treatments for Balsam and Concolor fir.

In conifers, stomatal closure is driven by a combination of reduced Ψ and increased hormones such as abscisic acid (ABA). Where water stress triggers responses in leaf cells to cause a decline in guard cell turgor, thereby reducing or closing stomatal apertures (Brodribb and McAdam 2013; Buckley 2005). In this experiment, fir species had a higher *gs* compared to white pine seedlings. In the combination of SxI, there were no significant differences on *gs* within each species except white pine, where increasing irrigation increased the *gs* values. Similar results were observed for net photosynthesis. In contrast to the fir species, white pine species close their stomata at an earlier stage thus creating a reduction in photosynthetic activities. Similar *gs* results were observed between Scots pine, Douglas-fir, and black pine (Zweifel et al. 2009). Similarly, the reduction of photosynthetic activities was reported in other studies (Ciais et al. 2005; Rennenberg et al. 2006; Granier et al. 2007; Reichstein et al. 2007) where *E* within each species also showed similar trends like *gs* and *A* (Li et al. 2003).

WUE had an opposite trend to the other photosynthetic parameters where white pine showed higher *WUE* compared to two fir species used in this experiment. Even though species did not differ with increasing irrigation levels, increasing irrigation regimes decreased the *WUE* for balsam and concolor fir seedlings. Plants can achieve larger *WUE* through either high *A* values, (Sinclair et al. 1984) low gs, or both. A greater *WUE* value indicated why white pine had a larger ratio between *A/E* as compared to the fir. However, high and medium stressed balsam and concolor fir seedlings had a slightly higher *WUE* compared to white pine seedlings within each irrigation level except for well-watered seedlings. Under water stress conditions, trees use water more efficiently to increase growth (Wright et al. 1993). Other studies have also shown that trees with higher *WUE* values show higher productivity or growth than trees with lower *WUE* under water stress condition (Jones 1993). Higher *WUE* often results from decreased *gs* and/or reduced leaf area (Zhang et al. 1997) which act as constraints for *A* and growth (Brendel et al. 2002).

This study found that fir species had a higher K^+ concentration in their foliar tissues as compared to white pine seedlings. Decreasing irrigation increased the K^+ concentration in general. Similar results have been reported for *Picea abies* (L.) and *Quercus rubra* L. (Thiec et al. 1995). Additionally, a study by Nilsen (1995) showed that increasing drought strain decreased the K^+ concentration in *Picea abies* (L.).

Calcium concentration significantly decreased with decreasing irrigation levels for each species. This shows Ca^{+2} is involved in the regulatory mechanisms in trees' responses to water stress due to an evoke increases in Ca^{+2} in guard cell by ABA (Bartels and Sunkar 2005) aligning with previous studies (Ma et al. 2005; Shao et al. 2008; Xu et al. 2013). Under SxI treatment, concolor and white pine showed the highest Ca^{+2} concentration that refers to a closure of stomata of these two species compared to balsam fir. This may make these two species more sensitive to water stress compared to balsam fir.

3.5. CONCLUSION

This study investigated various morphological and physiological parameters for three temperate climate conifer species under water stressed conditions. The goal was to increase the understanding of differences and similarities between white pine, balsam and concolor fir seedlings. Balsam fir seedlings responded to water stress with greater RRCD and RHG growth followed by concolor fir and white pine. White pine had a lower RRL compared to the fir species rejecting our hypothesis. Due to higher stem water potential and greater root development, the low drought-tolerant balsam fir seedlings took advantage of increasing plant growth. Under high water stress, white pine and balsam fir seedlings showed the more positive Ψ values where concolor showed the more negative Ψ values and are more susceptible to water stress. Additionally, white pine is able to use adaptation mechanisms to tolerate water stress, such as reducing stem water potential via closure of stomata, reduction of growth and cell enlargement, and limitation of gas exchanges. Due to these adaptations, the fir species showed higher gas exchange parameters; *A*, *gs* and a lower *WUE* compared to white pine. However, white pine seedlings quickly respond to water stress with closing their stomata thus leading a lower *A*. This may play an important role of having a higher *WUE* in terms of being a better drought tolerant species compared to fir species.

Concolor and balsam fir seedlings had a higher K^+ values and balsam fir had higher Ca^{+2} values followed by white pine. It is well-known, K^+ and Ca^{+2} play a vital role of signaling under water stress condition where increased K^+ concentration opens stomata (Thiec et al. 1995). This mechanism can explain to some degree drought tolerance in specific species (white pine and concolor species), and why these species may be more sensitive to stomatal signaling than low drought tolerant species.

We conclude that the three species tested showed various differences in terms of tree morphology and gas exchange parameters. White pine and balsam fir seedlings showed some similarities in terms of stem water potential under high water stress conditions. However, the physiological responses to water stress in conifer species is more complicated than just morphological responses. This implies there is likely a more direct link between genetic differences of species and responses to waters stress. Additional research looking at the genetic differences of conifer species is needed to explain the various morphological and physiological responses to water stress.

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CHAPTER FOUR

BIOMASS ALLOCATION AND NUTRIENT USE EFFICIENCY OF THREE TEMPERATE CONIFER SPECIES UNDER WATER STRESS

To be submitted to Annals of Forest Science.

ABSTRACT

Two-year-old (plug+1) containerized Balsam fir [*Abies balsamea* (L) Mill.] ‘Cooks’ (NY) and Concolor fir [*Abies concolor*] ‘Cibola’ (NM), and one-year-old bare-root Eastern white pine [*Pinus strobus*] (MI) transplants were grown under variable watering and fertilization with the goal of determining biomass allocation and nutrient use efficiency of these species in response to irrigation and fertilization. We hypothesized that white pine could have a greater tolerance to water stress compared to fir species due to their ability to develop a deeper tap root at an earlier age that may result in better water uptake, higher nutrient allocation, and better nutrient use efficiency. Relative root collar diameter, relative height, relative root length, dry shoot and root mass, root number and shoot/root (S/R) ratio were measured as growth parameters. Total nitrogen concentrations were determined for foliage, stems and roots to quantify nutrient allocation in these tissues. In addition, resource use efficiency parameters, such as assimilatory nutrient efficiency (ANUE), whole plant nutrient efficiency (NUE), index of nitrogen availability (N/RW) and root weight ratio (RWR) were calculated to determine the nutrient use of these species. Species varied ($P<0.05$) between two fir species and white pine seedlings in terms of stem water potential, growth parameters, biomass allocation and nutrient use. We observed that concolor fir had a greater capacity for consuming acquired water compared to white pine, leading to better above-ground growth and S/R ratio. The two fir species always exhibited higher nitrogen concentrations compared to white pine due to their better ability to tolerate drought and maintain their nutrient uptake under water stressed conditions. White pine plants had higher ANUE, NUE, RWR, and lowest N/RW compared to the two fir species, suggesting a better ability to tolerate water stress in this species.

Key words: Water stress, irrigation, fertilization, nutrient use efficiency, biomass allocation.

4.1. INTRODUCTION

Understanding the effects of water stress on plant growth and physiology has received a lot of attention from the scientific community for decades. Plants growing in a typical temperate forest have adequate precipitation to maintain soil moisture for supporting tree growth and survival (Gilliam 2016). Pine and fir species are among conifer species in temperate forests that have been extensively planted for their economic viability and growth characteristics around the world in places where they face challenges related to low precipitation and higher summer temperatures, resulting in water stress. Water deficit is recognized as one of the most crucial factors limiting growth and development of temperate trees in their natural habitat and/or their plantation areas (Granier et al. 2007). Ongoing increases in atmospheric CO_2 concentration are linked to warmer temperatures and are expected to increase the frequency and intensity of water deficit in trees (Meehl et al. 2007). However, the responses to water stress vary among tree species due to dependency on patterns of water use (Andrews et al. 2012). Under water stress, trees can develop some physiological adaptation functions to adjust to drought conditions to insure survival and growth (Chaves et al. 2002; Kozlowski and Pallardy 2002). Possible adaptive responses to water stress include processes such as reduction of leaf water content, loss of turgor pressure, closure of stomata, reduction of growth and cell enlargement, limitation of gas exchange (Jaleel et al. 2009), and reduction of photosynthetic pigments (Yordanov et al. 2000).

In addition to water, mineral nutrition plays a crucial role in fulfilling not only plant growth and development but also physiological functions (Groves et al. 1998). In general, evergreen foliage contains around 1-2 percent of N while the level of N in shoots and roots are around 0.5-1 percent N (Larcher 1995). However, the acceptable N concentration range is 1.3-3.5% N (dry weight) in conifers' foliage (Landis et al. 2010; Cregg 2005). The low nutrient requirement traits may provide an advantage to survive under extreme environmental conditions because of a high root-to-shoot ratio to reach available water and nutrients.

The plant analysis for nutrient diagnosis provides a useful quantitative relationship between plant growth, nutrient supply and mineral nutrients in the plant which is so crucial for optimum plant growth and development (Timmer 1991). Plant nutritional state varies in three phases, such as deficiency, luxury consumption, and toxicity (Timmer 1991). Timmer (1991) explains deficiency as restricting plant growth due to insufficient nutrient availability. Plants are stated in luxury levels when nutrient supplies level off, and nutrient levels are said to be in state of toxicity with tremendous increases. The relationships between nutrient content, nutrient concentration and plant growth and development provides useful information on the effects of under- and overfertilization on plants (Timmer 1991).

Under drought conditions, soil water deficit alters numerous steps of water transfer along the soil-root-leaf-atmosphere continuum that also may impact nutrient uptake and absorption by plants (Bréda et al. 2006). This process is vital for plants grown under intensive management because growers fertilize trees to accelerate growth and meet the plant’s nutrient requirements (Bilderback 1999). To alleviate the adverse effects of water stress, tree root systems play a critical role in water and nutrient transport, thus a desirable tree growth and development is provided (Alam 1999).

Roots are vegetative organs that not only anchor and stabilize trees in the soil but also deliver water and nutrients to growing trees (Fageria et al. 2016). In addition to providing building blocks for protein and nucleic acids, several essential elements are important in plant metabolism by acting as cofactor on activating enzymes under water stress (Nicholas 1975). Nutrient uptake is negatively affected by water stress (Alam 1999). Many studies have shown that nutrient uptake for K^+, Mg^{+2}, Ca^{+2}, Mg^{+2}, Fe^{+3}, Zn^{+3}, Cu^{+2}, N, P, and K are largely reduced by water stress (Alam 1999; Levitt 1980). The reduction of nutrients uptake especially nitrogen (N) caused by water stress limits plant growth and development (Nzokou and Cregg 2010). However, some elements such as N, P, Ca^{+2}, Mg^{+2}, Zn^{+2} and Mn^{+2} have shown increased uptake with increasing water stress (Singh and Singh 2004).

Root growth physiology is affected by a range of factors including species, water content, temperature, soil properties and other environmental factors (Kozlowski and Pallardy 2002). To reach available water and nutrients in the soil, plants are able to increase their root number and length even under water stress (Chaves et al. 2002). In addition to this, the root absorbing capacity plays an important role in the amount of nutrient uptake. For instance, some plants produce larger amounts of roots to establish in early years and then allocate more resources to aboveground tissues in later years (Kozlowski and Pallardy 2002).

Biomass allocation and the root-shoot ratio of the whole plant are often affected by lack of water (Passioura et al. 1993) and nutrients deficiencies (Kozlowski and Pallardy 2002). Tree species generally develop higher root-to-shoot ratios and deeper root systems in drier sites in comparison to the wetter sites (Kozlowski and Pallardy 2002) implying that the lower shoot-root ratio plants are less susceptible to water stress. Although plant growth rates usually decline when there is not enough available water in the soil, shoot growth is often more restricted compared to root growth (Nagarajan and Nagarajan 2010). Under water stressed conditions, root elongation and leaf area expansion decreased drastically at different rates (Bradford and Hsiao 1982). Leaf growth usually declines to a greater degree compared to root growth when root-to-shoot ratio increases due to photosynthate partitioning (Setter 1990). Under severe water stress conditions, some species are able to produce more roots to reach available water and nutrients in the deeper soil levels and resulting in better growth and development (Chaves et al. 2002).

Several studies pointed out that trees exposed to water stress develop higher root/shoot ratios than non-exposed plants in species such as *Pinus tadea* (Teskey et al. 1987), *Pinus pinaster* (Harfouchea 2003; Zas et al. 2004), *Pinus radiata* (Espinoza et al. 2014), and *Abies fabri* (Yang et al. 2013). Similar results have been found in some fir species (Gower et al. 1992). However, the growth and development patterns are different among those species. Although much is known about the mechanism of trees in general under water stress, few studies have focused on understanding the combined effect of irrigation and fertilization on the physiology and nutrient use efficiency in pine and fir species. The current studies investigate the physiological response to the combined effect of water stress and nutrition using white pine [*Pinus strobus* (L.)], balsam fir [*Abies balsamea* (L.) Mill] and concolor fir [*Abies concolor*] as model species due to economic importance, renewable resources, desirable color for Christmas tree production and landscaping.

We hypothesized that white pines should have a greater tolerance to water stress compared to balsam fir and concolor fir due to their ability to develop a deeper tap root at an earlier age. For example, in their native habitat, white pine trees usually develop multiple roots to spread outwards and downwards (Wendel and Smith 1990) while balsam and concolor fir develop a shallower root system (Frank 1990; Laacke 1990). Additionally, an increase in root length would provide for an increased adaptation for nutrient uptake under water stress in white pine compared to the two fir species. Specific objectives were (i) to investigate the differences and/or similarities in nutrient uptake and nutrient use efficiency between the three-conifer species; and (ii) to quantify morphological traits including rooting systems in relation to water stress response mechanisms observed in each species.

4.2. MATERIALS AND METHODS

4.2.1. Site description

This study was conducted in a greenhouse running east to west at the Tree Research Center (TRC) on the campus of Michigan State University, East Lansing, Michigan in 2015. The coordinates of the TRC are 42°65’N and 84°42’W. The greenhouse was covered with a double layer of clear plastic. The maximum temperature during the day and the minimum night temperature were about 33 °C and 18 °C, respectively throughout the experiment’s period (May 13th through September 15th). No artificial light was used for the experiment.

4.2.2. Plant material and containerization substrates

Two-year-old (plug+1) containerized Balsam fir [*Abies balsamea* (L.) Mill.] ‘Cooks’ (New York) and Concolor fir [*Abies concolor*] ‘Cibola’ (New Mexico), and one-year-old bare-root Eastern White Pine [*Pinus strobus* (L.)] (Michigan) transplants were obtained from a commercial nursery (Vans Pines nursery, West Olive, MI) in May 2015. Upon reception, seedlings were transplanted into black cylindrical 3-gal (11.2 liter) plastic containers with the potting mix consisting of Fafard 52 mix which contains Canadian sphagnum peat moss (30%), processed pine bark, perlite, vermiculite, wetting agents and dolomitic limestone, with a pH range of 5.5 to 6.5 after wetting (Conrad Fafard, Inc. Agawam, MA). The roots were pruned to approximately 15 cm before planting for all 168 seedlings used in this study. The initial diameter and height growth are shown Table 4.1.

4.2.3. Fertilizer treatment

The fertilizer used was a 5-6 month controlled-released formulation containing 15 % total nitrogen (N), 9 % of available phosphate (P_2O_5) (P) and 12 % of soluble potash (K_2O) (K) (Osmocote Plus 15-9-12, Everris NA Inc, Dublin, OH). The total nitrogen consists of ammoniacal and nitrate nitrogen with 7 % and 8 % content respectively. The fertilizer also contained 1% magnesium (Mg), 2.3% sulfur (S), 0.02% boron (B), 0.05% copper (Cu), 0.45% iron (Fe), 0.06% manganese (Mn), 0.02% molybdenum (Mo), and 0.05% zinc (Zn). The fertilizer was applied at one of two rates to each container: Low (FL) (4.5 g N/pot), and High (FH) (13.5 g N/pot). The fertilizer was applied at the beginning of the experiment through top dressing.

Table 4.1. Means (±SE) initial caliper and height, taken 13 May 20015, three conifer species grown in containers under three fertilizer rates and three irrigation levels combinations.

Species	Caliper (mm)	Height (cm)
Abies balsamea	4.51 (± 0.69)	22.13 (± 2.41)
Abies concolor	5.18 (± 1.03)	26.80 (± 2.72)
Pinus strobus	7.19 (± 0.91)	31.88 (± 7.14)

4.2.4. Irrigation treatment

Before irrigation treatments were applied, five specimens from each species seedlings were used to determine the plants water requirements according to the following procedure: Five containers were well-watered and allowed to drain gravitationally for 2 days. At the end of the 2-days, a volume of 1000 mL water was applied to each container and allowed to run off two hours. Then, the initial weight of the container was measured for each container. After two days, the final weights of the containers including the seedlings were measured and recorded. The difference between the initial and final weights of each containerized plant was assumed to correspond to the weight of the water utilized by the tree plus the evaporation from the container's substrate. This process was replicated 3 times to determine how much water each test seedling in the container used in a week.

Irrigation treatments were then established at 25, 50, and 100 % of the water requirement as determined above. Each irrigation level was increased by 10% every week to account for increases in water demands as trees grew during the experiment period. The three treatments corresponded to three weekly irrigation volumes: 1) low (750 ml), medium (1500 ml) and high (3000 ml) for balsam and concolor fir; 2) low (900 ml), medium (1800 ml) and high (3600 ml) for the pine seedlings. These volumes were applied manually throughout the experiment.

4.2.5. Stem water potential

Stem water potential (SWP) was determined on September 1, 2015 using the pressure chamber method with a plant water status console model 3115 (Soil Moisture Equipment Corp, Santa Barbara, CA) according to Turner (1988). Cuttings of about 5-10 cm were taken from the new growth. The samples were placed in the pressure chamber that was then pressurized. A measurement was taken when the cut surface was wet or shiny indicating xylem water coming back to the surface. Measurements were taken midday between 11:30 A.M – 2:30 P.M. Soil moisture content was measured (1:00-3:00 P.M) seven times during the experimental period in each pot for each treatment using a Field Scout TDR 300 Soil Moisture Probe (Spectrum Tech Inc, Plainfield, IL).

4.2.6. Morphological responses

Stem height, and root collar diameter (caliper) were measured at the beginning and end of the experimental period. Relative Height Growth (RHG) and Relative Root collar diameter growth (RRCD) were calculated as the difference between the end of season measurement and the initial measurement divided by initial measurement.

Additional growth parameters including, number of root (RN), dry shoot mass (DSM), dry root mass (DRM) and shoot/root ratio (S/R) [S/R = (DSM / DRM)] were measured or calculated.

4.2.7. Total nitrogen concentration on tissues (needle, stem, and root)

Current year tissue samples (needle, stem, and root) were collected on September 1, 2015 for analysis of nutrient concentrations. Tissues specimens were obtained from all plants in each treatment and mixed into a composite sample. Samples were stored at 4 ºC in sealed plastic bags and stored prior to processing and analysis. For processing, samples were oven dried at 65 ºC (± 5 ºC) for 48 hours, then ground into a fine powder with a kitchen mixer grinder. Samples were then acid-digested by the following process: Approximately 0.3 g of powder from each sample were mix with H_2SO_4 (4.5 mL) and H_2O_2 (1.5 mL) in a 100-mL digestion tube to pre-digest overnight. The digestion tubes were gradually heated in a digestion block (AIM600) to 340 ± 10 ºC (increasing 5 ºC every hour until 340 ºC) where they stayed at constant temperature for an hour. After the digestion was completed, tubes were filled with distilled water and placed on a lab mixer to homogenize the solution. To determine the total N concentration, an aliquot from each digest solution was analyzed for TKN on a SAN++ segmented flow analyzer (Skalar, Inc., Buford, GA, USA).

4.2.8. Resources use efficiency

The following resources use efficiency were calculated according to Sheriff et al. (1995):

- Assimilatory Nutrient Use Efficiency (ANUE): Foliar biomass production per unit of foliar N (g/mg/g).
- Nutrient Use Efficiency (NUE): Whole plant nutrient use efficiency in grams of biomass/g of N year (g/g).
- N/RW: Index of N availability (foliar N per unit of root weight) (mg/g).
- Root weight ratio (RWR): grams of root/g of total biomass (g/g).

4.2.9. Media nitrate (NO_3^-) and ammonia (NH_4^+)

Media samples were extracted from each specimen container using potassium chloride (KCl) and directly analyzed for NO_3^- and NH_4^+ concentrations on the SAN++ segmented flow analyzer (Skalar, Inc., Buford, GA, USA).

4.2.10. Data analysis

The experimental setup was a factorial design with 3 tree species (White pine, concolor fir, balsam fir), 3 irrigation levels [Low (Irr-1), Medium (Irr-2), High (Irr-3) per week], and 2 fertilization levels (Low and High). Treatment effects were tested using Analysis of Variance (ANOVA) for a 3 x 3 x 2 x 2 (replication) factorial. Each treatment had 5 seedlings for the fir species and 4 seedlings for the pine species totaling 168 individual trees. A level of significance of $\alpha=0.05$ was used for inferring any statistical significance. All variables were tested for normality using PROC TRANSREG, histogram and box-cox. Data that were not normal (RRCD, RRL, RN, DSM, DRM and S/R) were normalized using a log transformation. PROC MIXED was used to conduct analyses of variance (ANOVA) for all variables. When the model was significant, the Tukey's Honestly-Significant-Difference (HSD) method at $P \leq 0.05$ was used to make a pairwise comparison between responses. All statistical analysis were performed using SAS 9.4 statistical software.

The combination of irrigation and fertilization effects on foliar nutrient concentration, foliar nutrient content, and biomass production in 2015 were analyzed using vector diagrams as described by Haase and Rose (1995). Relative unit biomass was depicted on the z-axis when foliar nutrient content and foliar nutrient concentration were depicted on the x and y axis, respectively. Each data point was calculated and plotted relative to the initial (control) and adding fertilizer with irrigation regimes as an indication of relative magnitude and type of treatment response.

4.3. RESULTS

4.3.1. Stem water potential

Species (S), irrigation (I) and two-way interactions such as SxI, SxF (species x fertilization) affected Ψ ($P<0.05$). Under the interaction of SxI, the well-watered treatments that received high levels of irrigation maintained higher Ψ when compared to medium and low irrigation for each species. White pine and balsam fir seedlings showed higher Ψ compared to concolor fir under each irrigation treatment (Fig. 4.1). There was no difference between balsam and white pine under well-watered and medium watered treatments. However, white pine showed higher Ψ than balsam fir suggesting balsam fir was under more stress. Concolor fir seedlings had the lowest Ψ under low irrigation.

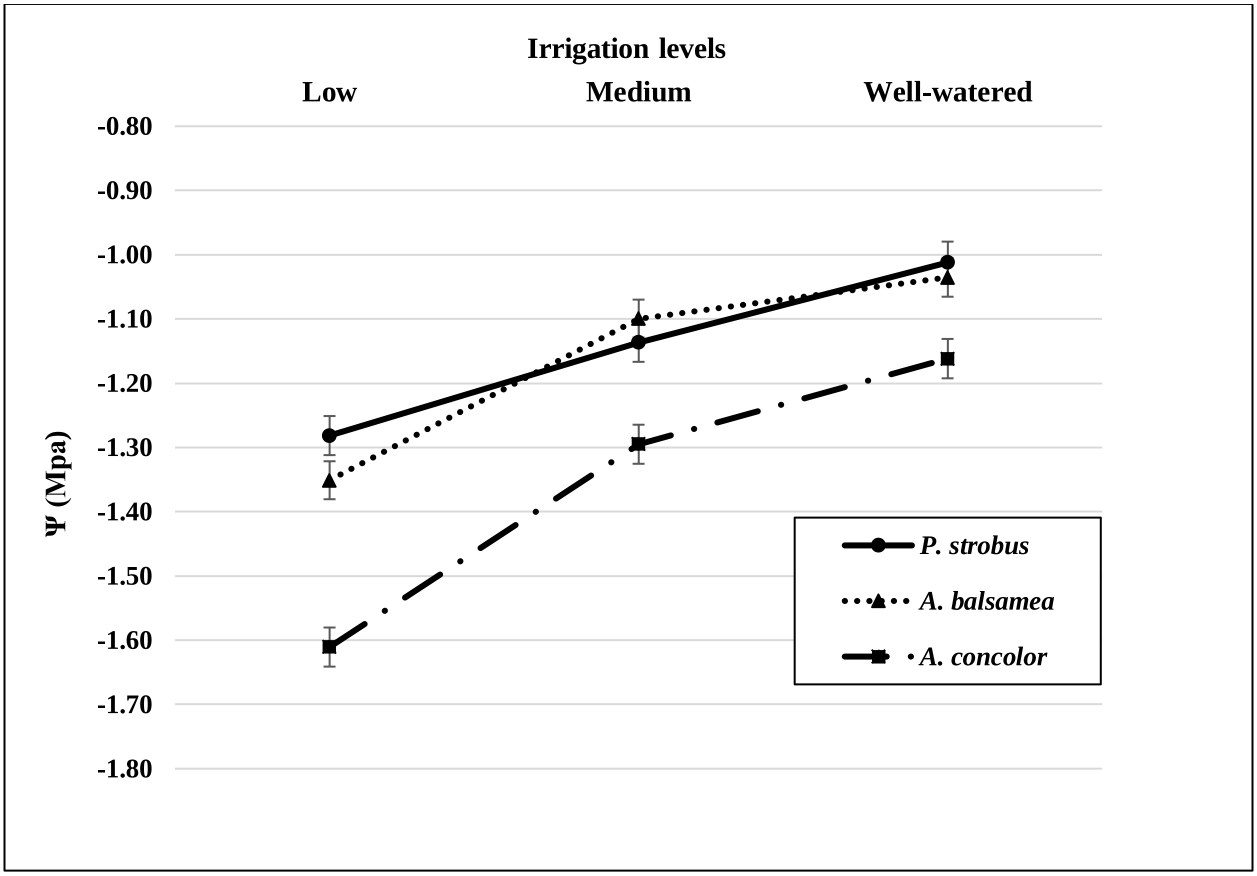

Figure 4.1. *Pinus strobus*, *Abies balsamea* and *Abies concolor* midday stem water potential (Ψ) (Mpa) of seedlings on September 1, 2015.

4.3.2. Soil moisture

With few exceptions (Data not shown), soil moisture content showed a trend similar to stem water potential. Balsam fir transplants had lower soil moisture compared to concolor fir and white pine for each irrigation regime suggesting balsam fir seedlings utilized more water. Increasing irrigation increased the soil moisture content for each species. The highest soil moisture content was observed on white pine pots under well-watered treatments on August 20th while the lowest soil water content was observed on balsam fir under low irrigated treatments on August 13, 2015.

4.3.3. Morphological features

4.3.3.1. Relative root collar diameter (RRCD) and relative height (RHG) growth

Concolor fir seedlings had the highest RRCD (0.47 mm/mm) followed by balsam fir (0.36 mm/mm) and white pine (0.18 mm/mm) (Table 4.2) when examining species as single factors. The white pine seedlings had the lowest RRCD growth whereas the concolor fir seedlings showed the highest RRCD for all fertilization rates. Well-watered and highly-fertilized balsam fir seedlings had the highest RRCD (0.62 mm/mm) while lowly irrigated and fertilized white pine seedlings showed the lowest values (0.12 mm/mm) (Table 4.3). Overall, increasing fertilization and irrigation rates increased the RRCD growth for all species. Data analysis indicated that there was significant effect of S, I, F as a single factor and the interaction between SxF and IxF on RRCD growth was also significant ($P<0.05$) (Table 4.3).

For Relative Height Growth (RHG), S, I, and F were all statistically significant ($P<0.05$) when examined as single factor (Table 4.3). Concolor seedlings had the highest RHG (0.30 cm/cm) followed by balsam fir (0.28 cm/cm). White pine seedlings had the lowest RHG (0.18 cm/cm) (Table 4.2). There was no significant difference between balsam and concolor fir seedlings in terms of RHG but increasing the irrigation regime increased the RHG. Increased fertilization also increased the RHG in general, but the interactions combinations for RHG were not statistically significant (P>0.05) (Table 4.3).

Table 4.2. Mean and standard errors of relative root collar diameter (RRCD), relative height growth (RHG), relative root length (RRL), root number (RN), dry shoot mass (DSM), dry root mass (DRM) and shoot: root ratio (S/R) measurements for three species under three irrigation and two fertilization levels.

	Species		
	Balsam fir	Concolor fir	White pine
RRCD (mm/mm)	0.36±0.01b	0.47±0.01a	0.18±0.01c
RHG (cm/cm)	0.28±0.01a	0.30±0.01a	0.18±0.01b
RRL (cm/cm)	4.48±0.11b	5.30±0.11a	3.34±0.11c
RN	92.05±12.76a	106.43±13.41a	48.93±13.11b
DSM (g)	10.39±0.66b	17.47±0.67a	19.41±0.69a
DRM (g)	8.09±0.49b	8.81±0.54b	17.02±0.54a
S/R	1.27±0.04b	1.97±0.05a	1.13±0.04b

Note: Number followed by the same letter in a row and species are not significantly different at p<0.05 using Tukey's adjustment.

4.3.3.2. Biomass

4.3.3.2.1. Shoot mass

Total DSM differed (P<0.05) between species, with balsam fir seedlings having the lowest dry (10.39 g) shoot mass compared to concolor fir seedlings (17.47 g) and white pine (19.41 g) (Table 4.2). The three-way interaction of SxIxF was also significant (Table 4.3). Irrigation and fertilization evaluated as single factor and all other potential interaction combinations were not statistically significant (P>0.05).

Table 4.3. Mean and standard errors, and summary analysis of variance for effects of relative root collar diameter (RRCD), relative height growth (RHG), relative root length (RRL), root number (RN), dry shoot mass (DSM), dry root mass (DRM) and shoot: root ratio (S/R) measurements for three species under three irrigation and two fertilization levels with alpha 0.05. *$p \le 0.05$ - **$p \le 0.01$ - *** $p < 0.0001$. ns: not significant.

Species	Irrigation levels	Fertilization (F)	RRCD (mm/mm)	RHG (cm/cm)	RRL (cm/cm)	RN	DSM (g)	DRM (g)	S/R
Abies balsamae	Low	4.5 g N/pot	0.25 ± 0.03	0.16 ± 0.03	4.67 ± 0.03	64 ± 30	10.55 ± 1.82	7.98 ± 1.17	1.24 ± 0.10
		13.5 g N/pot	0.34 ± 0.03	0.24 ± 0.02	5.67 ± 0.02	153 ± 28	10.86 ± 1.49	9.80 ± 1.11	1.09 ± 0.10
	Medium	4.5 g N/pot	0.29 ± 0.03	0.27 ± 0.03	4.61 ± 0.03	46 ± 32	8.40 ± 1.58	6.11 ± 1.17	1.49 ± 0.10
		13.5 g N/pot	0.36 ± 0.03	0.29 ± 0.02	5.19 ± 0.03	70 ± 28	10.90 ± 1.41	9.22 ± 1.17	1.18 ± 0.11
	Well-watered	4.5 g N/pot	0.31 ± 0.03	0.32 ± 0.03	4.49 ± 0.03	127 ± 37	11.84 ± 1.58	8.60 ± 1.33	1.35 ± 0.12
		13.5 g N/pot	0.62 ± 0.03	0.40 ± 0.03	4.66 ± 0.03	93 ± 32	9.78 ± 1.82	6.80 ± 1.17	1.27 ± 0.11
Abies concolor	Low	4.5 g N/pot	0.35 ± 0.03	0.24 ± 0.03	5.03 ± 0.03	129 ± 34	16.64 ± 1.69	8.83 ± 1.33	1.72 ± 0.11
		13.5 g N/pot	0.43 ± 0.03	0.22 ± 0.03	4.77 ± 0.03	70 ± 31	20.64 ± 1.69	9.29 ± 1.33	2.31 ± 0.12
	Medium	4.5 g N/pot	0.42 ± 0.03	0.31 ± 0.03	5.16 ± 0.03	143 ± 34	18.01 ± 1.69	10.56 ± 1.33	1.59 ± 0.12
		13.5 g N/pot	0.47 ± 0.03	0.30 ± 0.03	5.55 ± 0.03	88 ± 32	16.71 ± 1.49	7.94 ± 1.25	2.17 ± 0.11
	Well-watered	4.5 g N/pot	0.54 ± 0.03	0.34 ± 0.03	5.41 ± 0.03	116 ± 34	17.83 ± 1.58	8.51 ± 1.33	2.06 ± 0.12
		13.5 g N/pot	0.60 ± 0.03	0.40 ± 0.03	5.87 ± 0.03	93 ± 32	15.00 ± 1.69	7.73 ± 1.33	1.97 ± 0.11
Pinus strobus	Low	4.5 g N/pot	0.12 ± 0.03	0.13 ± 0.02	3.06 ± 0.03	43 ± 32	22.13 ± 1.82	21.11 ± 1.33	1.18 ± 0.11
		13.5 g N/pot	0.15 ± 0.03	0.14 ± 0.02	3.43 ± 0.03	68 ± 32	14.85 ± 1.58	13.19 ± 1.25	1.18 ± 0.11
	Medium	4.5 g N/pot	0.14 ± 0.03	0.14 ± 0.02	3.51 ± 0.03	37 ± 34	18.76 ± 1.69	18.33 ± 1.33	1.03 ± 0.11
		13.5 g N/pot	0.20 ± 0.03	0.20 ± 0.02	3.09 ± 0.03	58 ± 32	21.24 ± 1.58	19.93 ± 1.25	1.08 ± 0.11
	Well-watered	4.5 g N/pot	0.16 ± 0.03	0.21 ± 0.02	3.33 ± 0.03	46 ± 32	14.45 ± 1.82	12.94 ± 1.33	1.04 ± 0.11
		13.5 g N/pot	0.31 ± 0.03	0.26 ± 0.02	3.61 ± 0.03	43 ± 32	25.04 ± 1.69	16.59 ± 1.44	1.26 ± 0.11
	ANOVA	Species (S)	***	***	***	***	***	***	***
		Irrigation (I)	***	***	ns	ns	ns	ns	ns
		Fert. (F)	***	**	*	ns	ns	ns	ns
		S x I	ns	ns	ns	*	ns	ns	ns
		S x F	**	ns	ns	*	ns	ns	**
		I x F	*	ns	ns	ns	ns	ns	ns
		S x I x F	ns	ns	ns	ns	***	***	*

4.3.3.2.2. Root mass

Average total DRM values were 8.09 g, 8.81 g and 17.02 g for balsam fir, concolor fir and white pine, respectively (Table 4.2) when examining species as single factors. There were no significant differences ($P>0.05$) between balsam and concolor fir seedlings while white pine seedlings had a statistically higher DRM average value (Table 4.2). The three-way interaction between SxIxF was the only statistically significant ($P<0.05$) effect on the total DRM (Table 4.3).

4.3.3.3. Root related morphological features

4.3.3.3.1. Relative root length (RRL) and the number of roots (RN)

Relative root length (RRL) was significantly affected by S and F (Table 4.3). Concolor fir seedlings had the highest RRL (5.30 cm/cm) compared to balsam fir (4.48 cm/cm) and white pine (3.34 cm/cm) (Table 4.2). There was no significant difference between factors for RRL except for S and F. Likewise, the number of roots (RN) were significantly affected by S, SxI and SxF. White pine seedlings had the lowest RN (≅49) compared to balsam (≅92) and concolor (≅106) (Table 4.2).

4.3.3.3.2. Shoot / root ratio (S/R)

Species (S), the interactions between SxF, and SxIxF had a significant effect on S/R ($P < 0.05$) (Table 4.3). Concolor fir had the highest S/R ratio (1.97) followed by balsam fir (1.27) and white pine (1.13) when examining species as a single factor (Table 4.2), however, there was no significant differences between balsam fir and white pine seedlings. Low irrigation and high fertilization of concolor seedlings had the highest S/R ratio (2.31) while lowly fertilized and medium watering of white pine had the lowest values (1.04) (Table 4.3). Under each fertilization and irrigation level, concolor fir had the highest value compared to other species. There were no significant differences between white pine and balsam fir seedlings for all fertilization rates and irrigation levels.

4.3.4. Nitrogen Concentrations

4.3.4.1. Foliar tissues

Balsam fir foliar tissues had the highest nitrogen concentration (21.96 mg/g) followed by concolor (19.69 mg/g) and white pine (17.11 mg/g) (Table 4.4). Irrigation had no effect on foliar N concentration while fertilization treatments were statistically significant with high fertilization leading to higher N concentration in foliar tissues (Fig. 4.2[A]).

4.3.4.2. Stem

Concolor fir had the highest stem N concentration (6.68 mg/g) followed by balsam fir (5.81 mg/g) and white pine (4.77 mg/g). Well-watered plants had higher stem N concentration (6.47 mg/g) (Table 4.4), but medium and low watered trees had lower N concentration values (5.45 mg/g and 5.34 mg/g) with no significant differences between irrigation treatments. Fertilization treatments had statistically higher stem N concentrations for concolor and white pine seedlings, however, there was no effect on balsam fir specimens (Fig. 4.2[B]).

4.3.4.3. Root

Balsam and concolor had similarly higher root N concentration (12.20 and 11.63 mg/g) compared to white pine seedlings (9.92 mg/g) (Table 4.4). Well-watered specimens had higher root N concentration (13.16 mg/g) compared to lowly watered (10.36 mg/g) and medium watered (10.24 mg/g) plants, and there was no statistical difference between low and medium irrigation levels. The root nutrient concentration response to fertilization was statistically significant with highly fertilized plants having higher root N (12.36 mg/g) compared to lowly fertilized concolor fir and white pine (10.14 mg/g) (Fig. 4.2[C]).

Table 4.4. Mean, standard errors, and summary of ANOVA for effects of foliar tissue, stem and root nitrogen concentrations measurements for three species under three irrigation and two fertilization levels with alpha 0.05.

Species	Irrigation levels	Fertilization	Needle	Stem	Root
Abies balsamae	Low	4.5 g N/pot	19.68 ± 0.81	6.40 ± 0.51	10.69 ± 0.77
		13.5 g N/pot	24.24 ± 0.78	5.45 ± 0.51	11.81 ± 0.81
	Medium	4.5 g N/pot	20.16 ± 0.82	4.69 ± 0.53	10.19 ± 0.77
		13.5 g N/pot	23.00 ± 0.72	5.80 ± 0.50	11.13 ± 0.73
	Well-watered	4.5 g N/pot	20.69 ± 0.81	6.26 ± 0.51	14.16 ± 0.82
		13.5 g N/pot	24.00 ± 0.72	6.25 ± 0.51	15.21 ± 0.77
Abies concolor	Low	4.5 g N/pot	18.78 ± 0.78	4.83 ± 0.51	9.52 ± 0.82
		13.5 g N/pot	20.20 ± 0.81	6.68 ± 0.51	12.30 ± 0.77
	Medium	4.5 g N/pot	21.03 ± 0.82	6.52 ± 0.59	9.25 ± 0.82
		13.5 g N/pot	19.91 ± 0.78	6.90 ± 0.51	11.39 ± 0.82
	Well-watered	4.5 g N/pot	17.05 ± 0.78	7.03 ± 0.51	12.51 ± 0.82
		13.5 g N/pot	21.17 ± 0.82	8.13 ± 0.47	14.83 ± 0.77
Pinus strobus	Low	4.5 g N/pot	14.14 ± 0.81	3.58 ± 0.57	7.77 ± 0.87
		13.5 g N/pot	17.78 ± 0.78	5.09 ± 0.51	10.05 ± 0.82
	Medium	4.5 g N/pot	14.02 ± 0.78	3.69 ± 0.51	8.42 ± 0.82
		13.5 g N/pot	18.42 ± 0.78	5.07 ± 0.51	11.02 ± 0.82
	Well-watered	4.5 g N/pot	15.55 ± 0.81	4.49 ± 0.51	8.77 ± 0.82
		13.5 g N/pot	22.79 ± 0.81	6.67 ± 0.59	13.49 ± 0.94
	ANOVA	Species (S)	***	***	***
		Irrigation (I)	ns	***	***
		Fertilization (F)	***	***	***
		S x I	**	ns	ns
		S x F	***	*	ns
		I x F	*	ns	ns
		S x I x F	ns	ns	ns

Note: * p≤0.05 - **p≤0.01 - *** p<0.0001. ns: not significant.

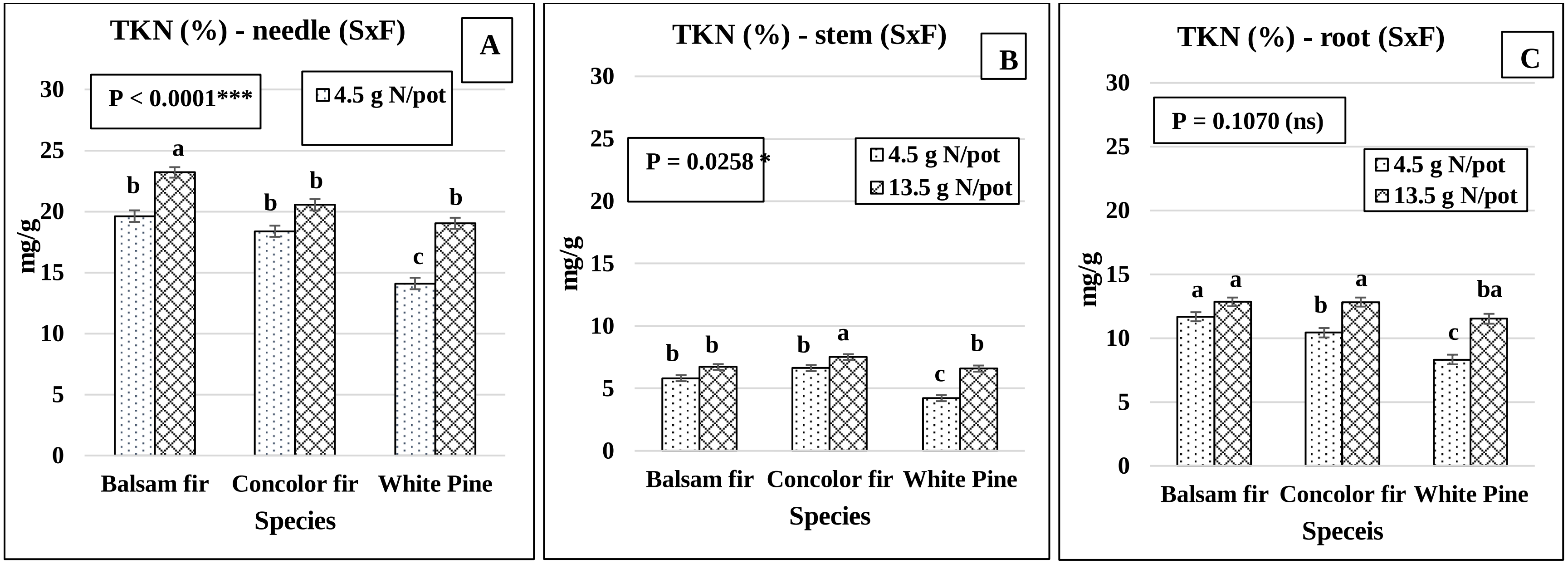

Figure 4.2. Total nitrogen concentration (TKN) on tree parts (needle (A), stem (B), and root (C)) for three species (Balsam fir, concolor fir and white pine) under fertilization treatments.

Note: Fertilization treatments: Low= 4.5 g N/pot, Hight= 13.5 g N/pot. ns= not significant, *p≤0.05, **p≤0.01, *** p<0.001. Graphs followed by the same letter indicates no statistical differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence).

4.3.5. Resources Use Efficiency

4.3.5.1. Assimilatory Nutrient Use Efficiency (ANUE)

White pine specimens had the highest *ANUE* value (1.20 g/mg/g) followed by concolor fir (0.92 g/mg/g) and balsam fir (0.48 g/mg/g). Low fertilization rate had higher *ANUE* (0.91 g/mg/g) compared to high fertilization rate (0.82 g/mg/g). The interaction of SxI was significant for *ANUE*. Increasing irrigation level increased the *ANUE* for balsam and concolor fir specimens, while white pine had the opposite trend. For SxF interaction, lowly fertilized white pine showed the highest *ANUE* (1.35 g/mg/g) and high fertilization rate of balsam fir showed the lowest values (0.45 g/mg/g). There is no clear trend for three-way SxIxF interactions (Fig. 4.3[A]).

4.3.5.2. Nutrient Use Efficiency (NUE)

Species when considered as single factor showed a trend similar *ANUE*. High fertilization had higher *NUE* (5.32 g/mg/season) compared to low fertilization (1.97 g/mg/season). For the SxIxF interaction, lowly fertilized plants had higher *NUE* for all species while increasing irrigation levels decreased *NUE* in some cases, others stayed similar for some of the treatment combinations (Fig. 4.3[B]).

4.3.5.3. Index of Nitrogen Availability (N/RW)

Species (S), irrigation (I), fertilization (F) as individual factors, and SxF and IxF interactions were all statistically significant for N/RW. Concolor fir had the highest *N/RW* (40 mg/g) followed by balsam fir (27.91 mg/g) and white pine (17.73 mg/g). Well-watered plants had the highest *N/RW* (31.07 mg/g) compared to low and medium watering treatments (26.73 and 27.77 mg/g respectively). High fertilization rate had the highest *N/RW* (31.36 mg/g) while low fertilization treatments had the lowest *N/RW* (25.69 mg/g). For SxF interaction, highly fertilized concolor fir had the highest *N/RW* (46.22 mg/g) and lowly fertilized white pine specimens had the

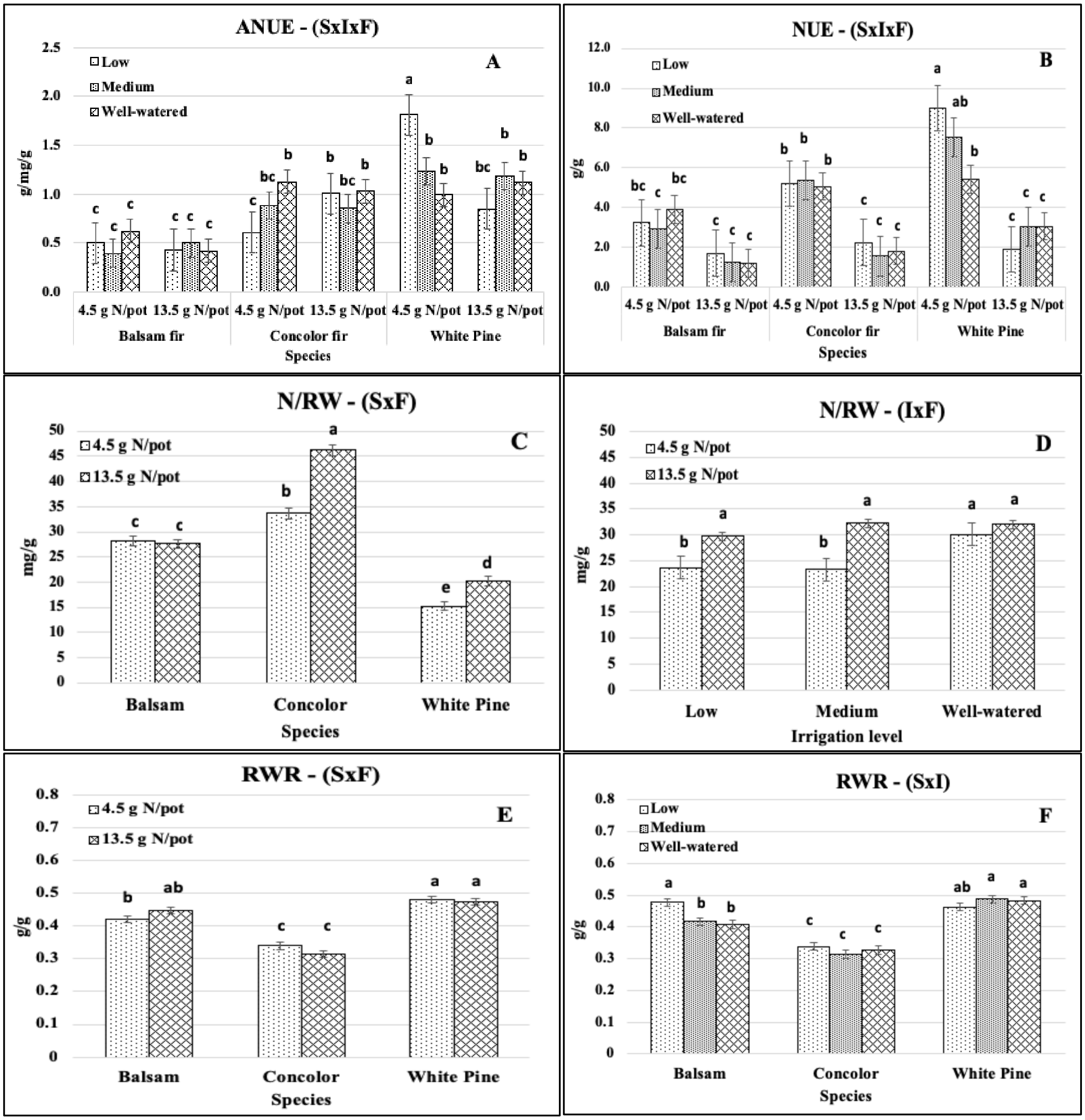

Figure 4.3. Resources use efficiency parameters with meaningful results in *Pinus strobus*, *Abies balsamea* and *Abies concolor* seedlings three irrigation regime and two fertilization rates.

Note: (A) ANUE, under the interaction of species (S) x irrigation (I) x fertilization (F); (B) NUE, under the combination of SxIxF; (C) NR/W, under the combination of SxF; (D) NR/W under the interaction of IxF; (E) RWR under the combination of SxF; (F) RWR under the interaction of SxI. Irrigation for fir species: Low =750 ml, medium=1500 ml, well-watered=3000 ml; for white pine: Low =900 ml, medium=1800 ml, well-watered=3600 ml weekly. Fertilization: Low= 4.5 g N/ pot and High= 13.5 g N/pot. The lower-case letter showed the statistical differences ($p \leq 0.05$) between and/ or within subject.

lowest *N/RW* (15.24 mg/g) [Fig. 4.3(C)]. There was no statistical difference between high and low fertilization rate for balsam fir. High fertilization rate showed no differences for well-watered treatment under the interaction of IxF (Fig. 4.3[D]). Well-watered irrigation regime had the highest *N/RW* compared low and medium irrigation under IxF interaction (Fig. 4.3[D]).

4.3.5.4. Root Weight Ratio (RWR)

Species (S), SxI and SxF were statistically significant for *RWR*. White pine had the highest RWR (0.48 g/g) compared to balsam fir (0.43 g/g) and concolor fir (0.32 g/g). Under SxF interaction, high and lowly fertilized white pine specimens had the highest *RWR* compared to high and low fertilization treatments in balsam fir and concolor fir (Fig. 4.3[E]). Under the interaction of SxI, increasing irrigation decreased *RWR* for balsam fir and there were no statistical differences between concolor fir and white pine species (Fig. 4.3[F]).

4.3.5.5. Vector Analysis

Vector diagrams indicated that the pattern of response in nutrient status differed noticeably between white pine and fir species. Vector analysis for all species indicated seedlings treated with the combination 2 nitrogen levels under 3 irrigation regimes had foliar N concentration and N contents higher than the seedlings in the beginning (initials or reference point) (Fig. 4.4, Fig. 4.5, Fig. 4.6). Observations of the direction of the change in relative foliar biomass revealed some specific trends. For instance, under the interaction of low irrigation with low and high fertilization and medium irrigated with low fertilized balsam seedlings were diagnosed as luxury consumption while other interactions were diagnosed as nutrient deficiency (Fig. 4.4). Concolor seedlings under all interactions of irrigation levels and fertilization rates were diagnosed as nutrient deficient (Fig. 4.5), while the whole interactions of the white pine seedlings were diagnosed as luxury consumption (Fig. 4.6). It is found that there is clear evidence of luxury N consumption in white pine compared to balsam and concolor fir. The nutrient concentration and content increased for all species compared to the reference. The combination of each irrigation level and fertilization rate, concolor fir had highest nutrient concentration, nutrient content, and relative unit dry weight while white pine showed the lowest values (Fig. 4.4, 4.5, 4.6).

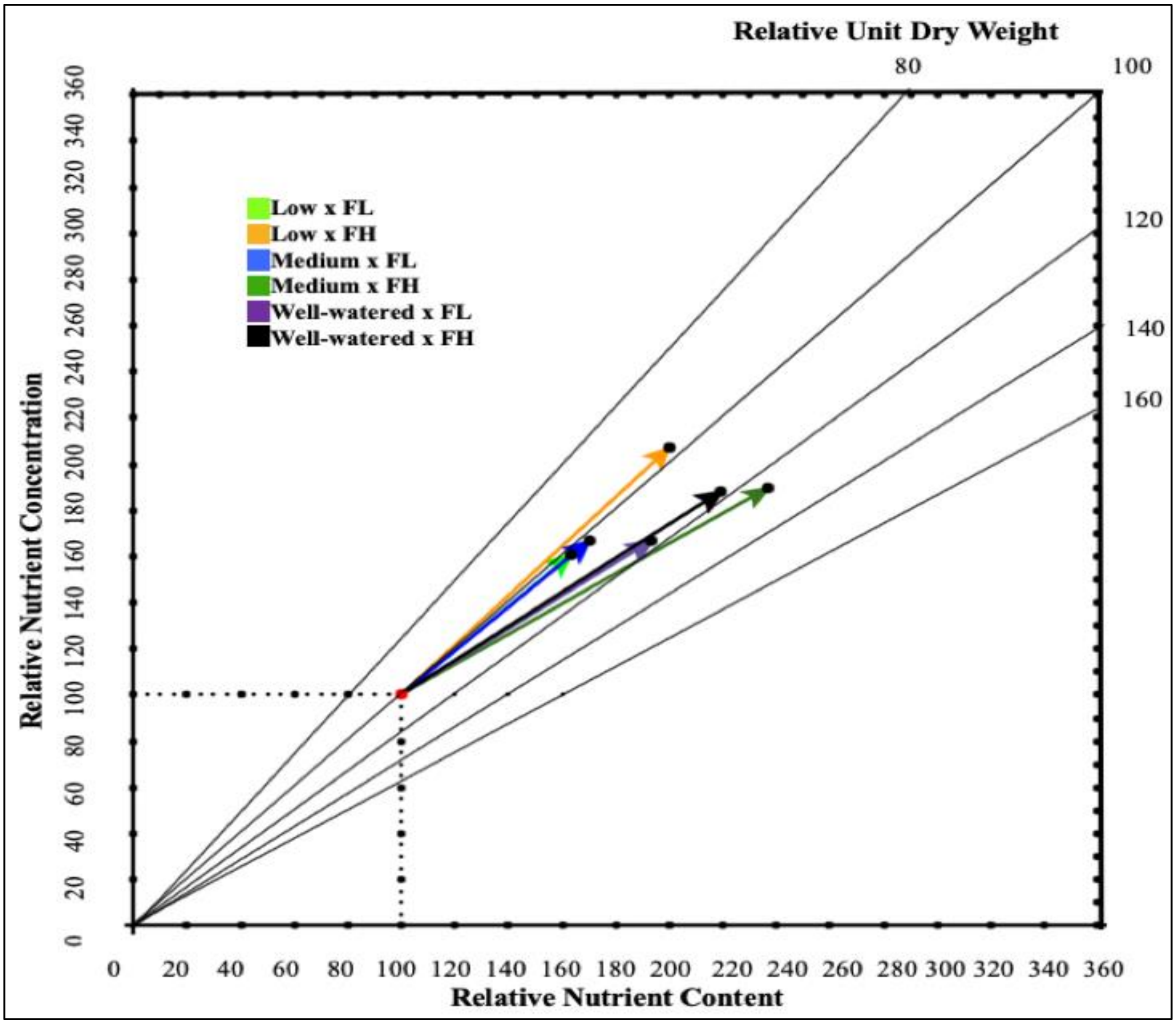

Figure 4.4. Vector analysis of needle N of *Abies balsamea* in response to the combination of fertilization rates and irrigation levels.

Note: Irrigation levels: Low= 25 %, Medium= 50 %, Well-watered= 100 % of water requirement; Fertilization rates: Low (FL)= 3.5 g N/pot, High (FH)= 13.5 g N/pot.

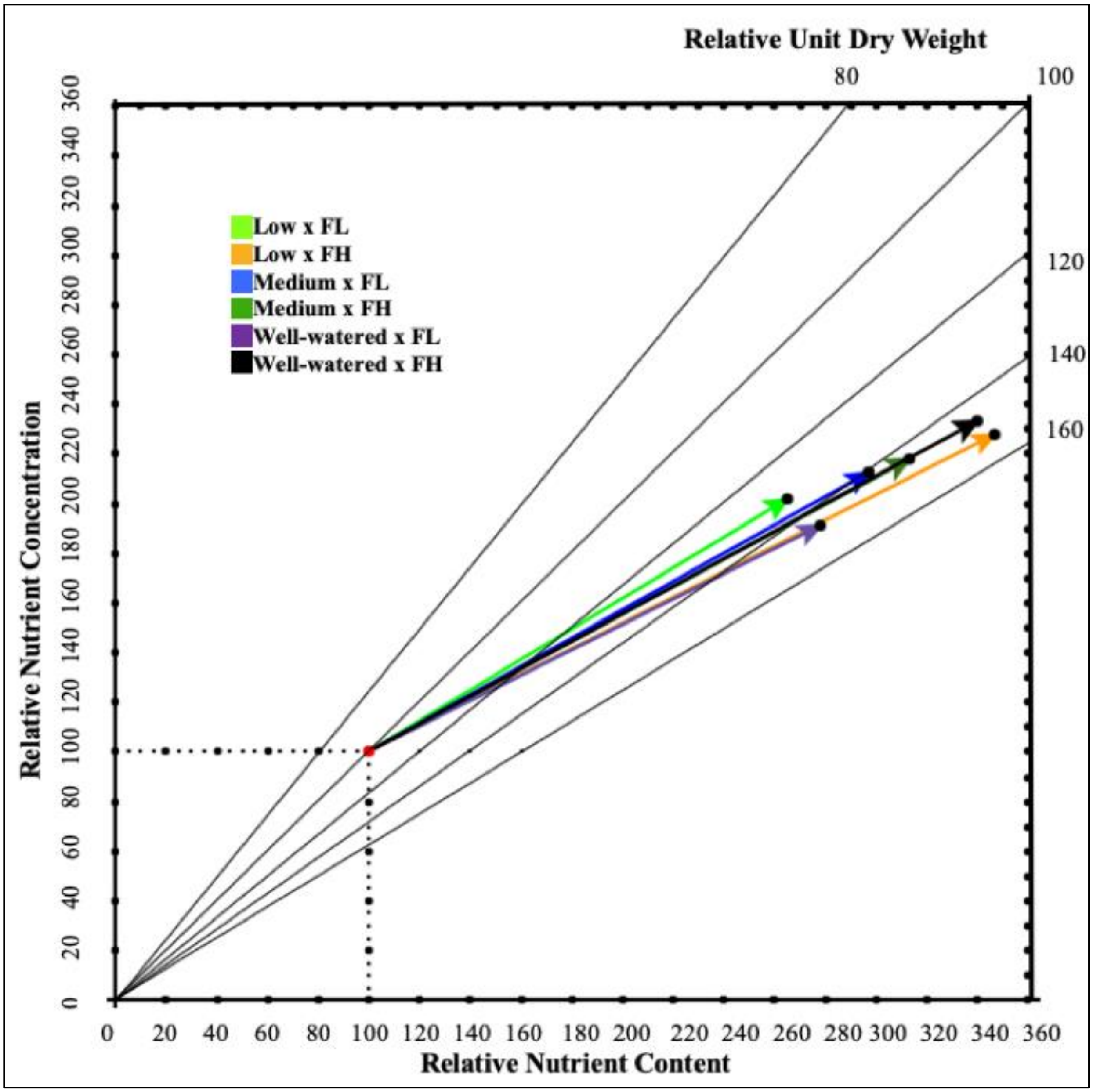

Figure 4.5. Vector analysis of needle N of *Abies concolor* in response to the combination of fertilization rates and irrigation levels.

Note: Irrigation levels: Low= 25 %, Medium= 50 %, Well-watered= 100 % of water requirement; Fertilization rates: Low (FL)= 3.5 g N/pot, High (FH)= 13.5 g N/pot.

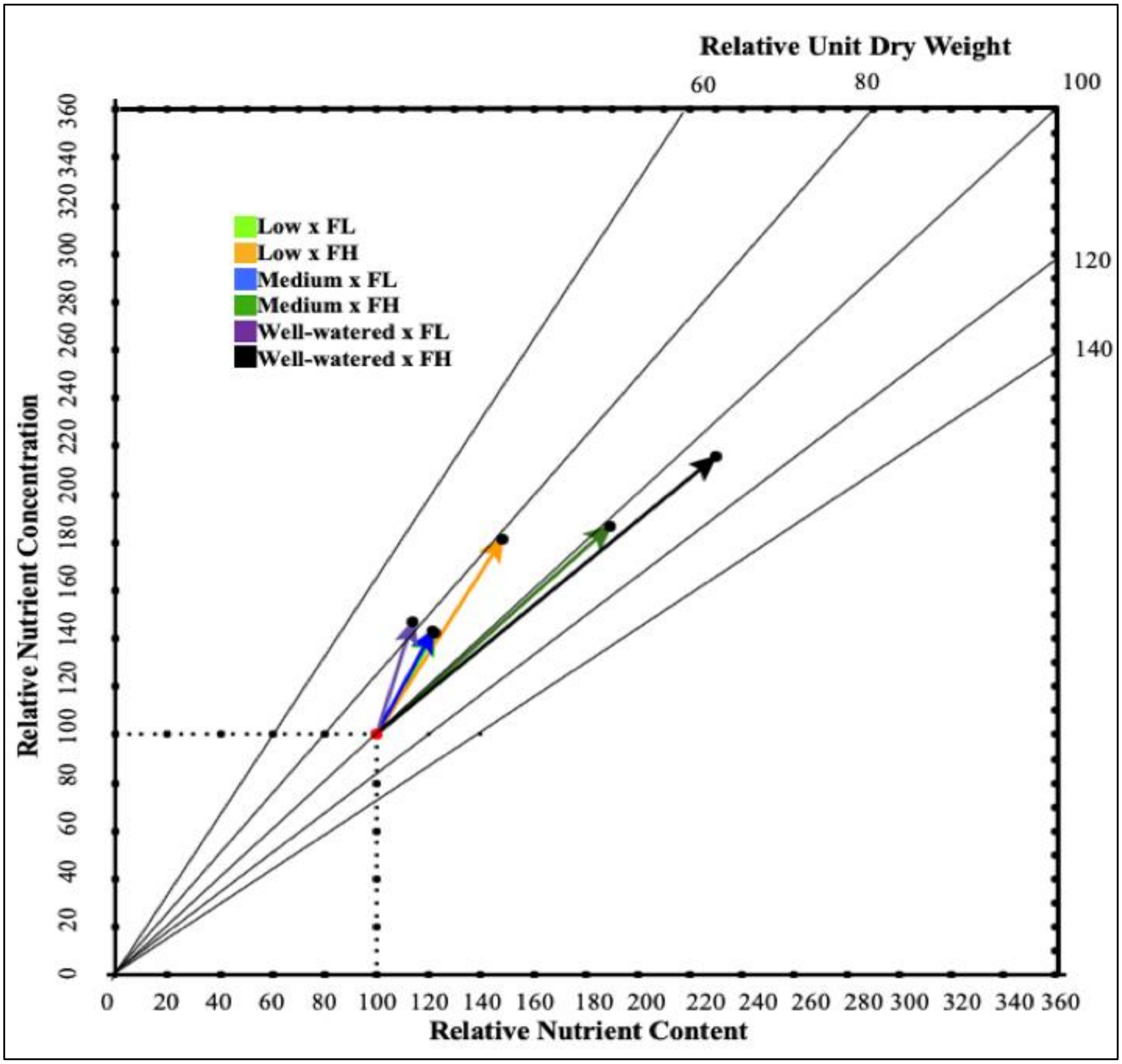

Figure 4.6. Vector analysis of needle N of *Pinus strobus* in response to the combination of fertilization rates and irrigation levels.

Note: Irrigation levels: Low= 25 %, Medium= 50 %, Well-watered= 100 % of water requirement; Fertilization rates: Low (FL)= 3.5 g N/pot, High (FH)= 13.5 g N/pot.

4.3.6. Soil nitrate (NO_3^- and ammonia (NH_4^+) analysis

For soil ammonia concentrations, S, I, SxI, IxF and SxIxF were all statistically significant. We observed that increasing irrigation increased soil ammonia concentration except in the case of highly fertilized concolor fir and white pine specimens where we found soil ammonia concentration to decrease under the interaction of SxIxF. Soil nitrate measurements were negligible and not considered for this analysis.

4.4. DISCUSSION

4.4.1. Plant water status and response

In this study, stem water potential (Ψ) was used as one of the main indicators of water status in plants. The differences in Ψ between plants is a product of the usage of water by plants and temperature due to linkage of increasing evaporation demand in the atmosphere when the vapor pressure deficit is raised in the middle of the day (Williams and Araujo 2002). In order to alleviate water stress, plants tend to close their stomata. This allows some plants to maintain their water potential at a high level resulting in better shoot growth (Kulaç et al. 2012). Concolor fir transplants had lower midday Ψ than the balsam fir and white pine, indicating that there were inherent differences in water relations characteristics between these species. Similar results were observed between Douglas-fir and lodgepole pine. These species had different patterns of midday Ψ (Andrews et al. 2012). This proves that species can differ from one to another in terms of adaptive mechanisms to water stress. It should be noted that pine species have a different leaf area compared to Douglas fir (Piñol and Sala 2000). These morphological traits might be another reason why concolor fir showed a lower Ψ compared to white pine and balsam fir. White pine has a stronger strategy of conservative control of stomatal conductance compared to fir species under severe water stress. In general, however, containerized seedlings experience less planting stress compared to bare-root seedlings due to stock type differences (Alm 1983; Nilsson and Örlander 1995). In addition, a previous study states that the conservative control of stomatal conductance is regulated by water loss for fir species such as Douglas-fir (Domec et al. 2008).

4.4.2. Effects on plant growth and biomass allocation

In general, growth and the root-shoot ratio of the whole plant are affected significantly by lack of water (Passioura et al. 1993) in line with this study. Plant and/ or shoot growth rates are often more restricted compared to root growth when there is not available water in the soil (Nagarajan and Nagarajan 2010). For instance, plants reduce the capacity to absorb water and nutrients from soil via their root system under drought stress (Alam 1999). It is not surprising that different species would develop different diameter and height growth responses because these variables are also controlled by the inherent genetic potential of the species. This has been demonstrated by several previous studies that have shown that under stress, *Pinus taeda* produced higher belowground biomass than other model species used in the study (Teskey et al. 1987) while Douglas-fir had higher aboveground biomass in another comparative study (Gower et al. 1992). In both studies, these two species were exposed to same growing conditions and differences in biomass production were largely attributed to species intrinsic genetic potential (Teskey et al. 1987; Gower et al. 1992). However, plant stock type selection can influence root growth in drier sites due to different growth capabilities (Grossnickle 2012) that containerized-grown seedlings may have a greater root growth during the first growing season (Becker et al. 1987; Burdett et al. 1984). The greater root growth can increase to uptake more nutrient via water by plants resulting in greater biomass allocation.

In our study, the growth and development patterns clearly differed between pine and fir species. The two fir species developed more growth (height and diameter) compared to the pine species. This is in line with other studies (Perry 1989; Lyr and Hoffmann 1967; Bongarten and Teskey 1987) which showed that pine species allocated resources to roots early to favor establishment, while fir species favor aboveground expansion with early shoot elongation and diameter growth (Cuny et al. 2012). *Pinus ponderosa* has been shown to be able to survive under drought stress conditions by developing a deep taproot that facilitates the acquisition of soil water (Fernández et al. 2014). On the other hand, *Abies pinsapo* species close stomata rapidly using abscisic acid (ABA) during water stress and maintain closed stomata if water stress continues (Sánchez-Salguero et al. 2015). Studies have shown that even closely related Scots pine (*Pinus sylvestris* L.), and black pine (*Pinus nigra Arn. subsp. Salzmannii (Dunol) Franco*) respond differently to water stress (Martínez-Vilalta and Piñol 2002; Sánchez-Salguero et al. 2012). The difference was related to the ability of black pine to withstand drought stress compared to Scots pine.

It has been previously reported that species are capable of developing mechanisms in response to drought. For instance, they have different abilities among species to acclimate to, and survive, environmental change reported to be mostly dependent on differences in hydraulic characteristics and patterns of water use (Allen et al. 2010). Additionally, xylem water potential can be buffered or slowly changed due to variations in a species stem water storage caused by increased transpiration and xylem water flux (Meinzer et al. 2009).

As expected, biomass allocation was very similar to the growth response. The DSM (above ground biomass) was significantly different between the three species. Additionally, white pine produced greater DRM (below-ground biomass) compared to the two fir species that may provide an advantage to the white pine of being more drought tolerant among this species. Trees may invest more growth towards below-ground biomass to optimize water uptake while minimizing water loss from transpiration (Brunner et al. 2015). Environmental conditions are one such reason that biomass (shoot and root) accumulation differs from species to species (Kozlowski and Pallardy 2002).

Tree root systems are responsible for water and nutrient uptake, and they play an important role for adaptations under water stress conditions. Roots not only may grow deeper into soil to reach available water, but may also act as sensors for shoots regarding water shortage conditions (Hamanishi and Campbell 2011). Root related features such as RRL, RN and S/R measured in this study as an indicator for biomass allocation response for each species showed higher RN for fir species compared to white pine indicating a better ability for the two *Abies* species to produce fine roots. The S/R ratio was also higher for the two fir species due to their superior production of above-ground biomass compared to white pine. Value of S/R were reduced by water stress as previously demonstrated by other studies for pine species (Herzog et al. 2014; McMillin and Wagner 1995; Setter 1990; Bongarten and Teskey 1987).

Biomass ratios are generally affected by drought stress (Akinci and Lösel 2012). Under stress conditions, root elongation and leaf area expansion decrease drastically but these two processes are not equally affected (Bradford and Hsiao 1982). Leaf growth usually declines to a greater degree compared to root growth when root/shoot ratio increases due to photosynthate partitioning (Setter 1990). It has also been reported that under severe stress conditions; some species may be able to produce more roots to reach available water and nutrient in the deeper soil level. This has been observed in *Pinus tadea* (Teskey et al. 1987), *Pinus ponderosa* (McMillin and Wagner 1995), *Pinus pinaster* (Harfouchea 2003; Zas et al. 2004), *Pinus radiata* (Espinoza et al. 2014), *Abies fabri* (Yang et al. 2013), and in several fir species (Gower et al. 1992). This may explain differences observed between the three model species for root morphological parameters.

4.4.3. Effect on nitrogen allocation and resources use efficiency

The foliar N concentrations observed in this study were generally higher than values obtained in stems and roots (Table 4.4). Increased fertilization positively affected not only foliar N but also stem and root N concentrations too. Irrigation treatments also generally positively affected all plants' organs. High growth and biomass accumulation acquired with higher irrigation regimes should normally create a greater demand for nutrients (Clarkson 1985), normally met by higher nutrient uptake in high fertilization treatments. Water stress also affects plant nutrition due to nutrient absorption from soil and transports to upper part of the tree via water. Plant nutrition is vital for plants grown under intensive management because farmers usually fertilize trees to accelerate growth and meet the plant's nutrient requirements (Bilderback, 1999). Among nutrients, nitrogen (N) is the most vital element for improved productivity. The shoot and root growth are affected by N fertilization and uptake for Fraser fir (Nzokou and Cregg 2010).

Nutrient uptake is negatively affected by water stress as it decreases the nutrient uptake by plants (Alam 1999). When plants are exposed to drought stress, essential nutrient elements take part in the metabolism to act as cofactor or enzymes activators (Nicholas 1975). Many studies show that nutrient uptake is mostly reduced by water stress (Levitt 1980), such as, K^+, Mg^{+2}, Ca^{+2}, Mg^{+2}, Fe^{+3}, Zn^{+3}, Cu^{+2}, nitrogen, phosphorus, and potassium. Some of these elements, such as nitrogen, phosphorus, Ca^{+2}, Mg^{+2}, Zn^{+2} and Mn^{+2} increase in some species with increasing water stress (Singh and Singh 2004). We observed higher N concentrations in fir organs compared to white pine, possibly explained by their higher RN that expanded their ability to uptake available nutrients.

Plant roots are capable of adapting to various environmental conditions to uptake water and nutrients for biological functions. However, under water deficit conditions, the flow of water and nutrient might decrease and/or restrict growth for some plants causing a negative effect on trees through such mechanisms as nutrient uptake and reduction in N use efficiency. In general, increased irrigation and fertilization have a positive effect on the efficiency of carbon assimilation leading to an increase in net primary productivity in plants (Nzokou and Cregg 2010). In this study, increased irrigation increased the *ANUE* while higher fertilization rate had a greater *ANUE* and *NUE*. It can also be assumed that assimilatory and non-assimilatory activities such as, biochemical processes, structure, and storage must have played a role in the resources partitioning process that should also be taken into account (Margolis and Brand 1990). Well-watered irrigation treatments enhanced the index of N availability (*N/RW*) compared to low and medium irrigation treatments suggesting that water stress had a negative effect on assimilation in plants used (Brunner et al. 2015).

Vector analysis demonstrated that almost all balsam and concolor seedlings indicated N deficiency while white pine seedlings indicated luxury consumption. However, both balsam fir and white pine indicated luxury consumption compared to concolor fir seedlings under water stress due to having a better strategy of conservative control of stomatal conductance and low transpiration. On the other hand, red pine showed the different magnitude of vector compared to white pine in the study (Timmer and Armstrong 1987). Nitrogen is considered the most responsive nutrient and provided positive shifts in mass which diagnosed N deficiency in black spruce (Haase and Rose 1995) in line with balsam and concolor fir. In the study, concolor fir was more responsive under each interactions of irrigation level and fertilization rates compared to balsam fir and white pine.

4.5. CONCLUSIONS

This study investigated the growth, root morphology, biomass allocation and resource use efficiency of three temperate conifer species under varying irrigation and fertilization treatments. Growth was enhanced by irrigation and fertilization treatments with significant differences observed between species. Different biomass allocation patterns were observed for fir and pine species with greater aboveground biomass and shoot to root ratio observed for the two fir species compared to white pine. The greater nutrient use efficiency observed in fir was attributed to the production of an expanded network of small roots (higher RN) developed under water stressed conditions in firs. Increases in aboveground biomass not only played a role in the partitioning of resources to assist root growth, but also played a crucial role in water stress responses. The three species in this study had different strategies for coping with water stress. Concolor and balsam fir developed a denser root system and were able to increase their ability to uptake water and nutrients therefore displaying a drought avoidance mechanism, while white pine transplants had a lower S/R ratio and higher below-ground biomass viewed as desirable traits for drought tolerance. The resource use efficiency analysis suggests that under low water stress and high fertilization rates, species have a greater efficiency in carbon accumulation causing an increase in overall biomass. Nitrogen was mostly allocated to the foliar tissue compared to root and stem tissues. Increased irrigation not only improved the resources use efficiency, but also improved other physiological characteristics. Further physiological studies are underway to confirm these preliminary results and examine other hypotheses related to water and nutrient use physiology of commercially important temperate conifer species.

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CHAPTER FIVE

THE EFFECT OF THE ALTITUDE OF PROVENANCE ON EARLY GROWTH AND PHYSIOLOGY OF TURKISH FIR (*ABIES BORNMUELLERIANA*)

To be summitted iForest.

ABSTRACT

Four-year-old Turkish fir [*Abies bornmuelleriana*] transplants were grown in semi-controlled conditions in hoop houses with three watering regimes (0.00, 1.25, and 3.70 cm/week) with the goal of determining the effect of seed source altitude on the growth and physiological responses of Turkish fir species to water stress. Morphological characteristics, including relative root collar diameter (RRCD), and relative height growth (RHG) and stem volume (d^2h) were measured. Water stress was monitored by measuring stem water potential (Ψ) on a subset of transplants from each treatment. Physiological variables include photosynthesis (A), stomatal conductance (gs), transpiration rate (E), internal CO_2/ambient CO_2 ratio (C_i/C_a), water use efficiency ($WUE=A/E$) and intrinsic water use efficiency ($WUEi = A/gs$), chlorophyll fluorescence (Fv/Fm) and carbon isotope discrimination rate ($\Delta^{13}C$) were determined. Altitude had affected RRCD, RHD, and stem volume with plants originating from seeds collected at higher altitudes generally performing better values than lower altitude seed sources. Well-watered seedlings had higher A, Gs, E, C_i/C_a, WUE, Fv/Fm, and lower $WUEi$ and $\Delta^{13}C$ compared to water-stressed transplants. Altitude affected only *WUEi* and was not significant for other physiological variables. Our study suggests that altitude of the provenance did not significantly affect the early plant physiology under water stress, with water availability in the soil playing a more crucial role for growth and development. However, we recognize that other physiological parameters such as bud break could be more sensitive to the provenance's altitude and should be more considered before a definite conclusion is made of the trends observed.

Key words: Water stress, Turkish fir, altitude, plant physiology, gas exchange.

5.1. INTRODUCTION

The Earth’s surface temperature has risen almost 1 °C over the past century due to climate change caused by elevated atmospheric greenhouse gases (IPCC 2014). A clear warming trend will continue in the future (IPCC 2014) that can negatively affect plant ecosystems, forest areas, as well as species and their habitats in temperate forests. For instance, global soil moisture will diminish due to continuous global warming and a substantial change in the precipitation pattern (Yang et al. 2013). Such changes in climate and global soil moisture are expected to influence altitudinal and latitudinal distribution of tree species.

Elevation change has a significant impact on both structural and functional traits of plants (Losso et al. 2016). The altitudinal and latitudinal distribution of plant species in their natural habitat has an impact on growth, seed production, and seedling establishment (Kelly and Goulden 2008). These altitudinal gradients are also useful for examining plant response to temperature because at high elevation, air temperature changes significantly across short distances (Körner 2007). This information is becoming increasingly important due to variation in climate patterns caused by climate change. At high elevations, due to temperature change and lack of water in the soil, trees are exposed to unusual stressors. In response to drought stress, plant species develop morphological and physiological strategies such as increased water absorption and transportation via root elongation (Bengough et al. 2011), declination of transpiration and maximization of CO_2 intake (Mwanamwenge et al. 1999), development of robust root systems (Sharp and Davies 1989), reduction in leaf number and development of smaller leaf area to lower transpiration rates (Maes et al. 2009). Plants have also been shown to sometimes have decreased stomatal size (Cutler et al. 1977) and increased stomatal density (Zhang et al. 2006) as a response to stress. These anatomical and morphological adjustments are due to physiological and biochemical mechanisms (stomatal regulation: ABA accumulation-stomatal closure; proline content; photosynthesis decreases) developed to maximize water uptake and minimize water loss.

It is unclear how these responses are affected by the altitude in their natural habitat due to changes in temperature and soil moisture in future climate change scenarios. In general, the water supply of the trees is abundant at higher altitudes compared to lower altitudes however, increasing altitude increased acclimation to drought (Li et al. 2004). Water stress varies widely with altitude and the adaptation and acclimation to drought conditions play a crucial role for tree growth and survival (Charra-Vaskou et al. 2012). For instance, water stress occurs when precipitation is low and evaporation is high at lower altitudes (Charra-Vaskou et al. 2012). Morphological and physiological parameters are affected by altitudinal differences under water stress conditions. The specific leaf areas, growth, root-to-shoot ratio, and carbon isotope composition were different for two *Picea* seedlings (Li et al. 2004) when photosynthesis, carbon balance, and conductance differed for *Pinus flexilis* at different altitudes (Reinhardt et al. 2011).

In the last decade, scientists have been using an instantaneous measure of photosynthetic parameters such as net photosynthetic rate, stomatal conductance, transpiration rate, and carbon isotope discrimination as an explanation of plant growth and productivity under environmental stresses, such as water stress. Moreover, carbon isotope discrimination ($\Delta^{13}C$) performs as an integrated profile of the cumulative tree water stress throughout the period of active growth. Carbon isotope discrimination not only varies among species (Zhang and Marshall 1994) but may also vary among provenances that provide a better explanation of genetic variation due to well-designed foliage structure (Zhang and Cregg 1996). Therefore, this technique has been used to examine plants and whether they adapt to new environments or not.

Turkish fir [*Abies* bornmulleriana] is naturally found from Kızılırmak (Halys) Valley in northern Anatolia to Mount Uludağ in the Western Black Sea region (Anonymous, 2006). Turkish fir are economically important species not only in Turkey but also in Europe and the United States due to their desirable traits in ornamental horticultures, Christmas trees and as landscape plants (Sevik 2012). As a consequence, Turkish fir has been has been widely planted for Christmas tree production and as a landscape tree around the world (Tumen et al. 2010). However tree plantations and reforestation programs are sometimes established in areas where these plants face environmental changes related to water availability and temperature (Sevik 2012).

It is well established that under severe environmental conditions conifer populations can develop morphological and physiological adaptations to environmental stresses. I hypothesize that variation in altitude and provenance in Turkish fir species would significantly affect early growth and establishment success due to their evolution and adaptation to environmental stresses in their natural habitats. In this study, Turkish fir seedlings produced from seeds collected at two elevations from two provenances were compared for their responses to water stress.

The objective of the study was to determine morphological and physiological differences as affected by the altitude of origin in this economically important species. Results obtained could be used to guide the selection process for material to be used in plantation and reforestation programs in arid areas of Turkey and around the world.

5.2. MATERIALS AND METHODS

5.2.1. Site description

This study was conducted in hoop houses running east to west at the Tree Research Center (TRC) on the campus of Michigan State University, East Lansing, Michigan in 2017. The coordinates of the TRC are 42°65’ N and 84°42’ W. The hoop houses were covered with a transparent polyurethane tarp to keep rainfall out of the treatment plots. In addition, to prevent lateral movements of soil moisture between experimental plots and to maintain the integrity of each irrigation treatment, each hoop houses was divided into five treatment sections using oriented strand board wrapped with a plastic cover vertically inserted about 1.1 m into the ground. All hoop houses were completely opened at their east and west ends, and up to about 20 cm from the ground on their south and north sides to allow free airflow.

5.2.2. Irrigation treatment

Each of the hoop house sections was randomly assigned one of the three irrigation treatments with all five sections replicated in each of the three hoop houses (blocking) in a randomized complete block design. Each hoop house had a zero irrigation and two medium and high irrigation treatment sections for a total of three treatments within five sections in each block. Irrigation water was supplied through drip system equipped with an automated controller Netafim^{TM} (Netafim Irrigation Inc., Fresno, CA) throughout the growing season (1 May to 15 September). The layout and tubing were designed to simultaneously apply water to all three replicated plots for each irrigation level (0, 1.25, and 3.75 cm/week) which corresponded to 0, 126 and 266 min of run time per week. The irrigation application rates were determined based on precipitation rates from their natural provenances, and the numbers averaged and rounded for a better application.

5.2.3. Plant material

One-year-old (plug+0) containerized Turkish fir [*Abies bornmuelleriana*] transplants were received from a commercial nursery and put in ground for three seasons (2014-2016) at the TRC at Michigan State University. The sourcing nursery had raised these large-scale transplants as a part of the Collobrative Fir Germplasm Evaluation (CoFirGE) project developed collaboratively by Michigan State University, North Carolina State University, Washington State University, and Pennsylvania State University to evaluate Turkish fir genetic sources as potential new species for the United States Christmas tree industry. The two sources selected were from two provenances (Adapazari and Karabuk in Turkey) approximately 145 km apart (Fig. 5.1). The low and high altitudes at Adapazari varied from 1024-1309 m (AL) to 1448 m (AH), while at Karabuk varied from 1030-1321 m (KL) to 1349-1472 m (KH), respectively (Table 5.1). The average temperature and yearly rainfall in their natural habitat are 14.5 °C and 838 mm, and 13.4 °C and 490 mm for Adapazari and Karabuk, respectively (Fig. 5.2.).

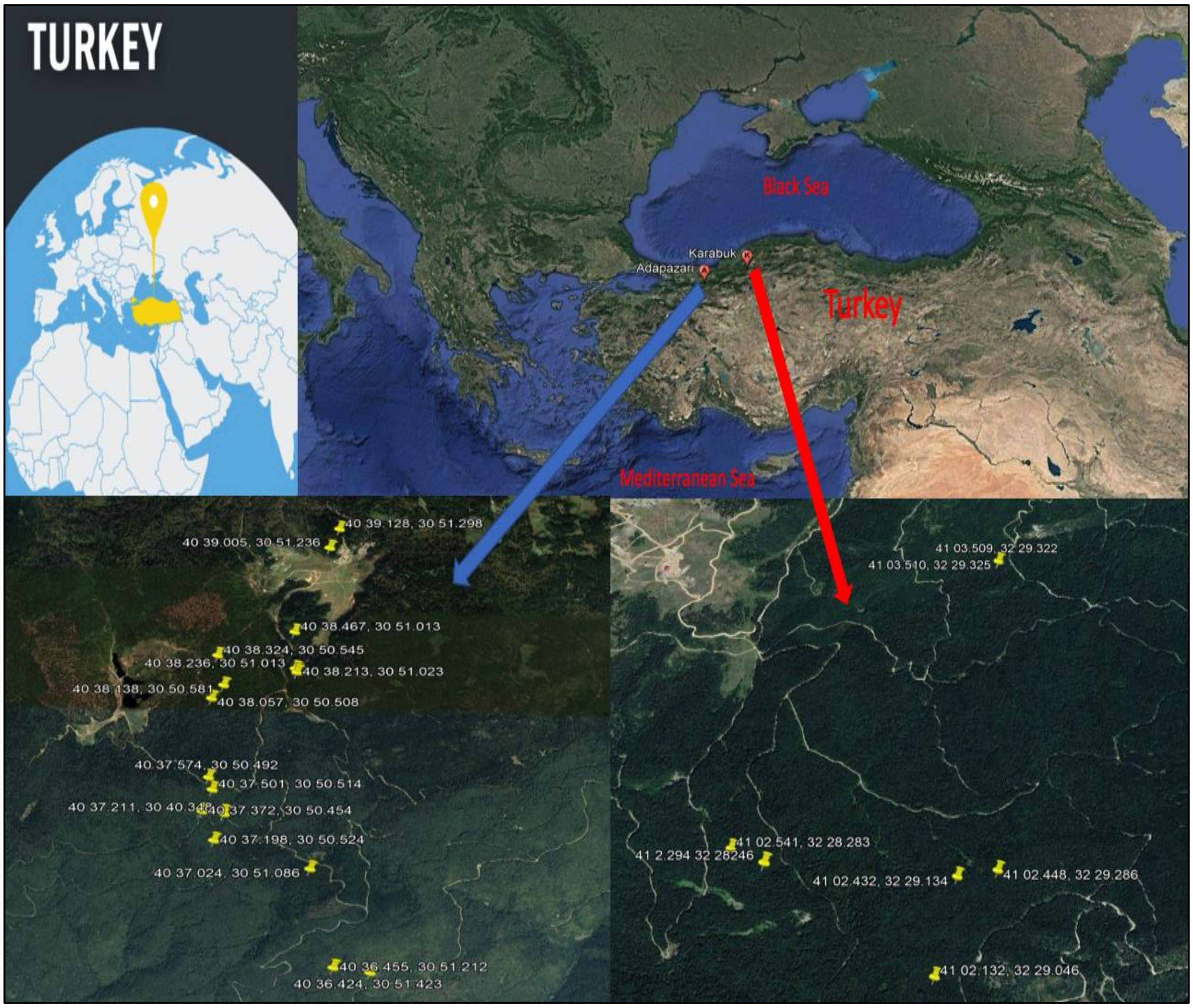
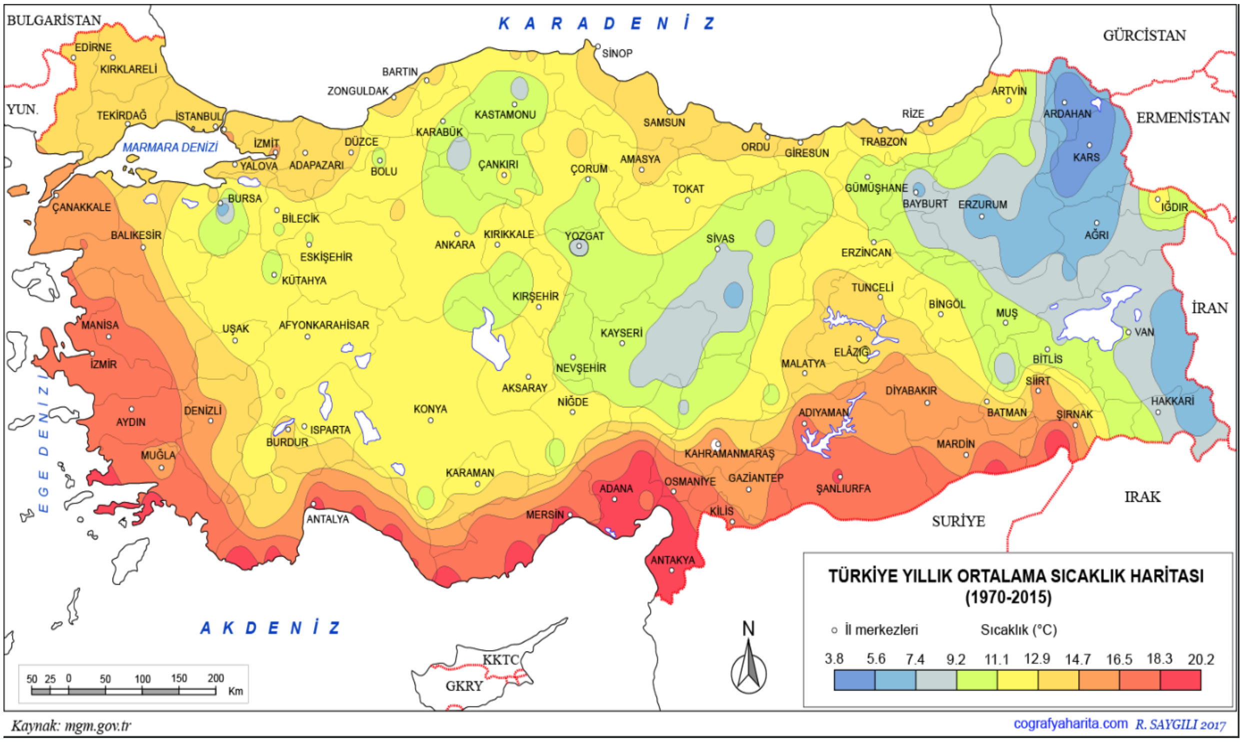


Figure 5.1. The coordinates of seed sources of Turkish fir from Turkey (Google Earth Pro).



Kaynak: mgm.gov.tr

cografyaharita.com R. SAYGILI 2017

Figure 5.2. The map for mean temperature conditions in Turkey between 1970-2015 (Obtained from mgm.gov.tr).

In 2015, plants were fertilized with 10 g of a controlled-released formulation containing 20 % nitrogen (N), 10 % available phosphate (P_2O_5) (P) and 10 % of soluble potash (K_2O) (K) (The Andersons, Inc, Webberville, MI) at the beginning of the growing season. Throughout the 2015 and 2016 growing season, plants were well-watered throughout the growing period (May to September) to maintain healthy tree growth. In the spring of 2017, transplants were installed in hoop house for the watering experiment. Each hoop house contained 5 plots with 20 trees (5 rows and 4 trees per row) for each provenance (0.6 m spacing) for a total of 100 trees in each block.

Average seedling diameters and heights before transplanting were 21.7, 20.3, 17.5, 24.2 mm diameter and 49.8, 48.1, 46.5, 52.3 cm height for Karabuk Low (KL), Karabuk High (KH), Adapazari Low (AL) and Adapazari High (AH), respectively. Weeds were controlled by hand or by applying a mixture of glyphosate (50 mL of glyphosate dissolved in 2-gallon water) using a CO_2-powered back-pack sprayer.

Table 5.1. Turkish fir (*Abies bornmuelleriana*) provenances and site characteristics.

Label	Provenance	Elevation range (m)	ID	Elevation (m)	Latitude	Longitude	Number	Total
AL	Adapazari	Low elevation (1024-1309)	41	1024	40-36-42.4	30-51-42.3	21	108
			42	1052	40-36-45.5	30-51-21.2	8	
			45	1117	40-37-02.4	30-51-08.6	5	
			47	1160	40-37-14.5	30-50-49.6	19	
			49	1258	40-37-35.2	30-50-58.3	34	
			50	1287	40-37-37.2	30-50-45.4	1	
			51	1309	40-37-50.1	30-50-51.4	20	
AH	Adapazari	High elevation (>1400)	60	1448	40-39-12.8	30-51-29.8	107	107
KL	Karabuk	Low elevation (1030-1321)	38	1030	41-03-50.9	32-29-32.2	7	100
			39	1032	41-03-51.0	32-29-32.5	12	
			40	1040	41-03-47.6	32-29-33.6	4	
			36	1302	41-02-43.2	32-29-13.4	7	
			35	1321	41-02-44.8	32-29-28.6	70	
KH	Karabuk	High elevation (1349-1472)	29	1349	41-02-49.1	32-28-41.0	10	97
			27	1360	41-02-56.6	32-28-33.7	20	
			28	1360	41-02-53.0	32-28-34.5	17	
			22	1382	41-03-04.4	32-28-28.9	4	
			21	1401	41-02-54.1	32-28-28.3	12	
			23	1423	41-02-45.5	32-28-22.3	3	
			24	1442	41-02-36.0	32-28-27.8	12	
			25	1472	41-02-26.1	32-28-25.8	19	

5.2.4. Morphological responses

Stem height (HG) and root collar diameter (RCD) growth were measured at the beginning and at the end of the experimental period. Relative Height Growth (RHG) and Relative Root collar diameter growth (RRCD) were calculated as the difference between the end of season measurement and the initial measurement divided by the initial measurement. Relative stem volume indices for each tree were calculated by multiplying the square of the diameter by height (d^2h) for the growing period.

5.2.5. Stem water potential

Stem water potential (Ψ) was determined eight times (July 07, July 17, July 27, August 02, August 10, August 16, August 23, August 31) in the growing period of 2017 using the pressure chamber method with a plant water status console model 3115 (Soil Moisture Equipment Corp, Santa Barbara, CA) according to Turner (1988). Four representative trees in each plot of two hoop houses were selected for water potential measurements (96 total for all plots each measurement time). Cuttings of about 5-10 cm were taken from the new growth. The samples were placed in the pressure chamber that was then pressurized. These measurements were taken midday between 11:30 A.M – 2:30 P.M. Soil moisture content was measured seven times during measurement period in each pot for each treatment using a Field Scout TDR 300 Soil Moisture Probe (Spectrum Tech Inc, Plainfield, IL).

Soil moisture content was taken at 10 different location in the same irrigation plot using a Field Scout TDR 300 Soil Moisture Probe (Spectrum Tech Inc, Plainfield, IL).

5.2.6. Gas exchange measurement

Photosynthesis was measured on four randomly selected trees from each treatment (total 144 transplants) using a LI-COR (LI6400XT, Lincoln, NE) conifer chamber with a RGB light source (6400-18A). The instrument was matched before each measurement and the reference CO_2 was maintained at 400 µmol, and the air flow rate was held at 500 µmol s^{-1}. Three readings were taken on current year developed branches on each tree between 11:00 AM and 3:30 PM. Measured shoots were cut after each measurement and stored in a cooler at ±5 °C. All gas exchange parameters were expressed on the projected needle area basis. Needles were removed from the shoot, images of all the needles were captured with a flatted scanner, and ImageJ software was used for image analysis (Rashband 2012). The measured needle area for each specimen was entered as section in the LI-COR system for adjustment of gas exchanges parameters previously measured in the hoop house for the specimen.

Several physiological parameters including the net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), substomatal CO_2 concentration (C_i), net CO_2 assimilation rate versus calculated substomatal CO_2 concentration (A/C_i) curves and internal CO_2/ambient CO_2 ratio (C_i/C_a) were determined from this process. Water use efficiency ($WUE = A/E$) was calculated as the ratio of carbon gain to water loss (Sinclair et al. 1984). The intrinsic water use efficiency ($WUEi = A/gs$) was calculated as the ratio of A to leaf conductance (gs).

5.2.7. Chlorophyll fluorescence (F_v/F_m)

The chlorophyll fluorescence was measured three times (July 27, August 10 and August 29, 2017) using a Hansatech Plant Efficiency Analyzer (PEA) Model PEA KM2, from Hansatech Instrument Ltd. (Kings Lynn, U.K.). Chlorophyll fluorescence measurements were conducted midday between 12:00 and 14:30 hour for each of the sampling dates. Needles were placed into a clip and acclimated dark for 15 minutes to allow all electrons acceptors to fully oxidize. The clips were then placed under the PEA to measure the F_v/F_m parameter. Four trees were measured in each plot under each irrigation x provenance x altitude combination for a total of 96 samples. Due to time and logistical limitations F_v/F_m was evaluated on two replications only at each sampling dates.

5.2.8. Carbon isotope discrimination

In late September 2017, we collected needle samples for stable carbon isotope analysis. For each treatment, we collected four samples (shoot) in upper branches of trees from current year growth. Samples were dried at 65°C (±5 °C) for 72 hours, then needles were separated from stems, and ground with a coffee grinder. The ground samples were separated using a mesh (0.420 mm) and packed in tin capsules for shipment to the UC-Davis Stable Isotope Facility in California for carbon isotopic analysis. Analysis was conducted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon LTD., Cheshire, UK). Final values from isotopic analysis were expressed in δ13C (‰), relative to international standards VPBD (Vienna PeeDee Belemnite) and air carbon.

We used the δ^{13}C ratio to calculate carbon isotope discrimination [Δ^{13}C (‰)], using the simplified equation (Farquhar et al. 1989)

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \quad (2)$$

where δ_a is the isotopic composition of air, assumed to be -8‰, and $\delta_p$is the isotopic composition of the plant material δ13C.

5.2.9. Data analysis

The experimental setup include one tree species (Turkish fir [*Abies bornmuelleriana*]) from 2 provenances (Adapazari and Karabuk), 2 altitudes (Low and High), and 3 irrigation levels [High (3.70 cm/week), Medium (1.25 cm/week), Zero(0.00 cm/week) per week]. Data were tested for homogenity of variance and normality and analyzed for mean comparison by analysis of variance (ANOVA). Data that were not normal (RRCD, d^2h, SWP, soil moisture, *E*, *WUE*, *WUEi*) were normalized using a log transformation, and a square transformation (*Ci/Ca*, *Fv/Fm*). A level of significance of $\alpha = 0.05$ was used for inferring any statistical significance. When the model was significant, the Tukey's Honestly-Significant-Difference (HSD) method at $P \leq 0.05$ was used to make a pairwise comparison between responses. All statistical analysis were performed using SAS 9.4 statistical software.

5.3. RESULT

5.3.1. Morphological features

Relative root collar diameter (RRCD) was significantly affected ($P<0.05$) only by irrigation and seed source elevations. Severe-stressed seedlings showed the lowest RRCD growth (4.50±0.45 mm/mm) compared to mild-stressed trees (5.77±0.31 mm/mm) and non-stressed trees (5.44±0.31 mm/mm) while there were no significant differences between non-stressed and mild-stress trees (Table 5.2). Altitudinal differences were significant on RRCD in that trees from higher altitudes showed higher RRCD (5.74±0.29 mm/mm) compared to lower altitudes (4.74±0.31 mm/mm). We did not observe any other differences between and within subjects ($P>0.05$).

Relative height growth (RHG) was significantly affected only by altitudinal differences ($P<0.05$) and plants originated from higher altitude showed higher RHG (15.24±0.61 cm/cm) compared to lower altitudes (12.51±0.63 cm/cm). Other factors between and within subjects did not show any statistical differences on RHG ($P>0.05$).

Stem volume was significantly affected by altitudinal differences and provenance x altitude interactions ($P<0.05$). Trees obtained from higher altitudes showed higher values compared to lower altitudes when altitude is a single factor. Under the interaction of provenance and altitude, trees from lower altitudes in Adapazari showed lower values compared to other provenance x altitude interactions (Table 5.2) and there were no significant differences between these interactions ($P>0.05$).

5.3.2. Stem water potential

Stem water potential (Ψ) was significantly affected ($P<0.05$) by irrigation (I), time (T), provenance (P) x time (PxT), IxT, altitude (A) x time (AxT), IxPxT, PxAxT, and IxPxAxT. Treatments that received high levels of irrigation maintained higher Ψ (-1.40 Mpa) when compared

Table 5.2. Mean and standard errors of relative root collar diameter growth (RRDG) and relative height growth (RHG) and mean of stem volume (d^{2}h).

		Provenances				
		Adapazari		Karabuk		
RRDG (mm/mm)	Irrigation level	Low	High	Low	High	Average
	3.70 cm/wk	5.31±0.62	6.38±0.62	4.94±0.61	5.14±0.62	5.44±0.31 ab
	1.25 cm/wk	5.39±0.63	6.06±0.61	5.69±0.66	5.93±0.61	5.77±0.31 a
	0.00 cm/wk	2.61±1.00	5.85±0.91	4.51±0.85	5.05±0.85	4.50±0.45 b
	Average	4.44±0.45 b	6.10±0.42 a	5.05±0.41 b	5.37±0.40 a	
RHG (cm/cm)	3.70 cm/wk	10.65±1.37	18.11±1.30	15.02±1.30	16.65±1.32	15.04±0.66
	1.25 cm/wk	11.29±1.35	13.79±1.30	15.36±1.32	13.99±1.27	13.61±0.65
	0.00 cm/wk	9.29±1.94	15.95±1.87	13.43±1.80	12.96±1.80	12.91±0.93
	Average	10.41±0.91 bB	15.86±0.88 aA	14.60±0.86 aA	14.53±0.86 aA	
d2h (cm3)	3.70 cm/wk	451.87	1076.52	668.70	745.20	735.57
	1.25 cm/wk	500.57	721.18	628.68	705.66	639.02
	0.00 cm/wk	144.19	474.12	427.66	545.12	397.77
	Average	365.54 bB	757.28 aA	575.02 bAB	665.32 bAB	

Note: Adapazari high altitude (>1400 m); Adapazari low altitude (1000-1309 m); Karabuk high altitude (1349-1472); Karabuk Low altitude (1000-1321m). Number followed by the same lower-case letter indicates no statistically significant differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence) under single factor. [c] Number followed by the same capitalized letter indicates no statistically significant differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence) under the interaction of provenance*altitude.

to medium stressed (-1.59 Mpa) and severe stressed trees (-1.83 Mpa). Provenances, altitude and their interactions had no statistical differences on seedlings Ψ (P>0.05). Stem water potential generally decreased until mid-August, and then increased throughout experiment period. The lowest Ψ was measured in August 16, 2015 (-1.71 Mpa) while the highest Ψ was measured in August 23, 2015 (-1.40 Mpa) due to decreased temperature and rainy week. Under the interaction of irrigation x altitude, there were no significant differences between altitude under each irrigation treatment except medium-watered Karabuk lower altitudes showed higher Ψ compared to higher altitudes (Fig. 5.3). Increasing irrigation level increased the Ψ for both provenances and altitudes (Fig. 5.3).

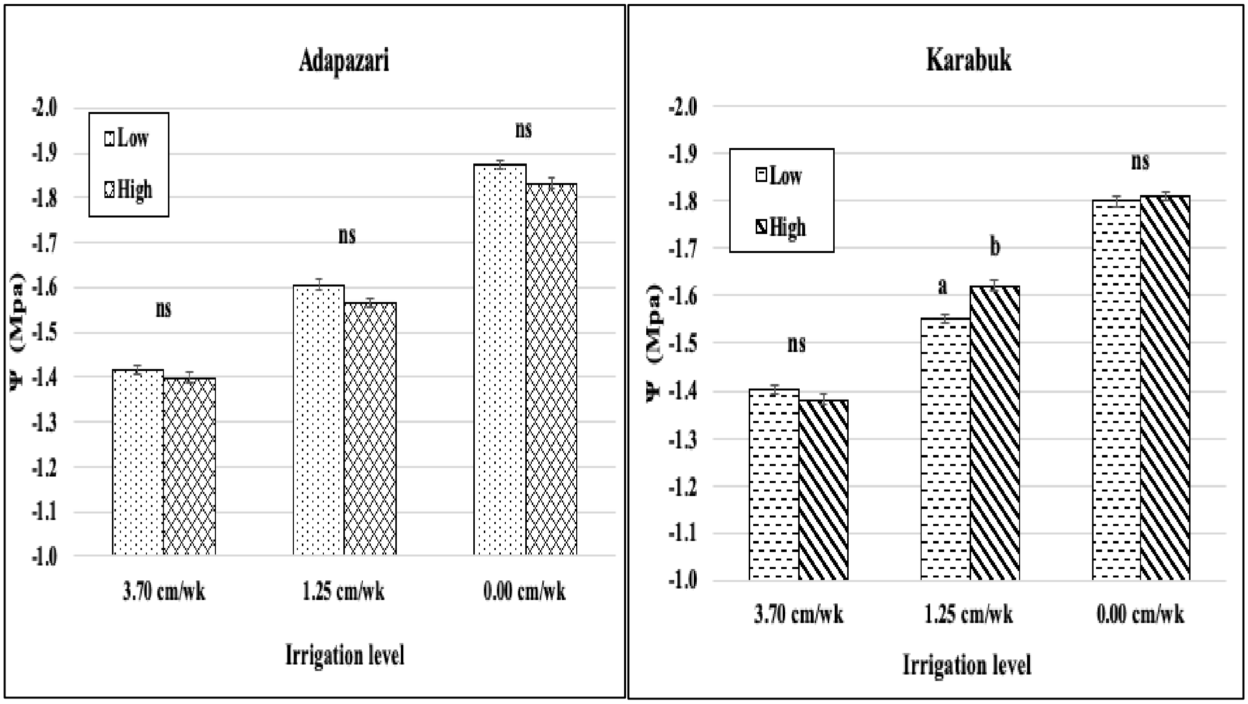


Figure 5.3. Mean and standard errors of midday stem water potential (Ψ) (Mpa) in Turkish fir species under the interaction of irrigation x altitude under each provenance separately in 2017.

Note: Graphs followed by the same letter indicates no statistical differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence). ns: not significant.

The relationship of stem water potential and net photosynthesis (*A*) under the interaction of altitude and elevation were shown in Fig. 5.4. The seedlings from AL had higher correlation (R^2: 0.32) than AH (R^2: 0.16) while KL had the lower correlation (R^2: 0.13) than KH (R^2: 0.16) (Fig. 5.4). When all altitude and elevations were combined, the correlation was R^2: 0.16.

5.3.3. Soil moisture

Increasing irrigation increased the soil moisture content for each measurement dates. Soil moisture had a highest value on July 7, 2017 and dropped the lowest point on July17, 2017 and then there was an increasing trend throughout the growing season except on August 24th.

5.3.4. Gas exchange and physiological parameters

The significance of gas exchange and physiological parameters are shown in Table 5.3.

5.3.4.1. Net photosynthesis (A)

Well-watered (3.70 cm/wk) trees had higher net photosynthetic rates (*A*) (7.47 µmol m^{-2} s^{-1}) followed by medium stressed (1.25 cm/wk) trees (6.61 µmol m^{-2} s^{-1}) and severely stressed (0.00 cm/wk) trees had lowest A (5.24 µmol m^{-2} s^{-1}). Trees from Karabuk provenance had higher *A* values (6.60 µmol m^{-2} s^{-1}) compared to Adapazari provenance (6.28 µmol m^{-2} s^{-1}). The interaction of IxPxA was significant ($P=0.036$) that well-watered plants from Karabuk higher altitudes had the highest values while severe-stressed trees from Adapazari low altitudes had the lowest values (Table 5.4). However, altitude had no statistical differences as a single factor ($P>0.05$).

5.3.4.2. Stomatal conductance (gs)

The general trend for *gs* was similar to *A* except for IxPxA interactions where well-watered trees from Karabuk higher altitudes had the highest values (0.10 mol m^{-2} s^{-1}) while severe-stressed trees from Adapazari lower altitudes had the lowest values (0.05 mol m^{-2} s^{-1}) (Table 5.4).

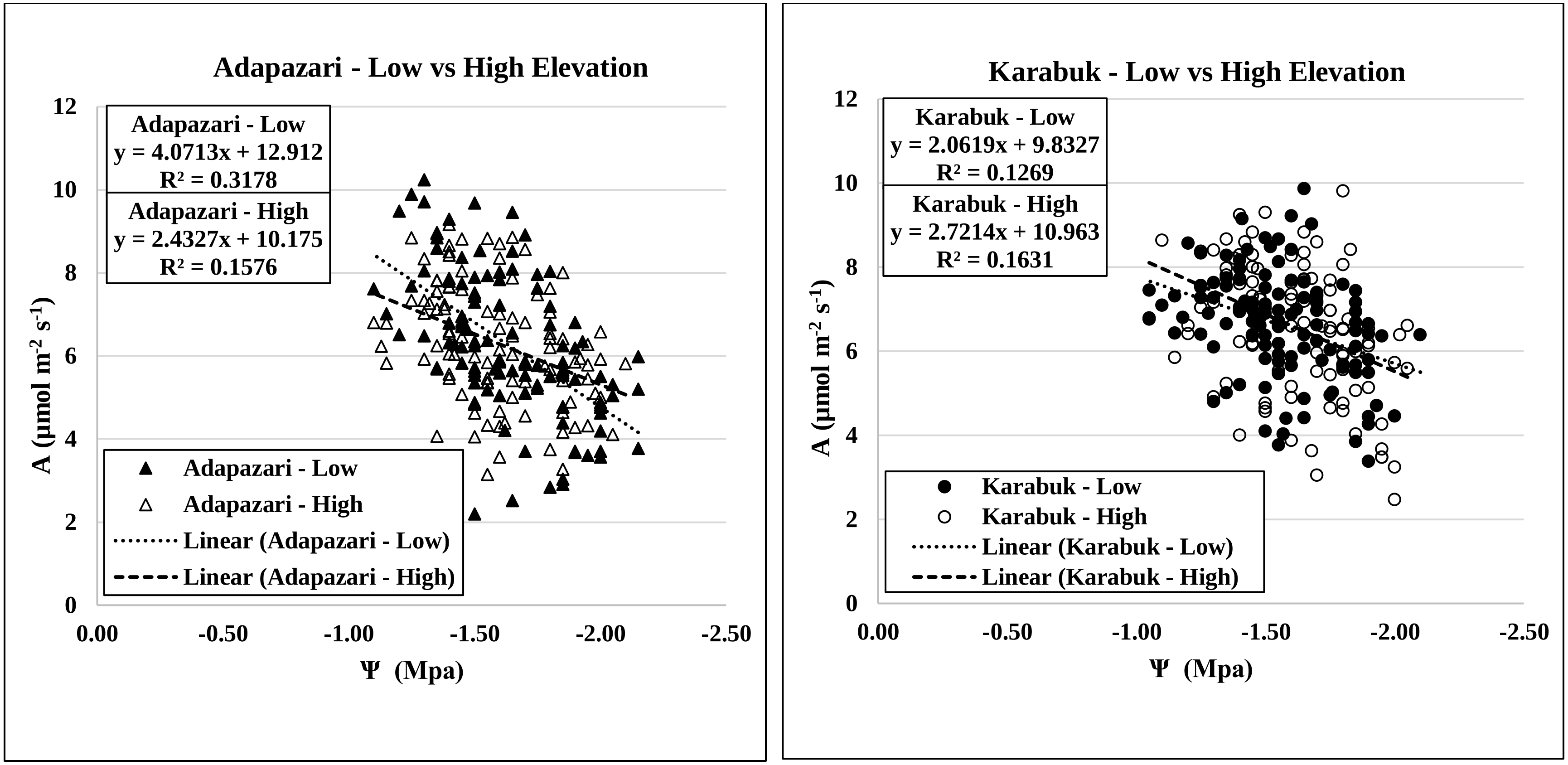


Figure 5.4. Relationship between *A* and Ψ in *Abies bornmuelleriana* seedlings from low and high altitudes of Adapazari and Karabuk provenances.

Table 5.3. Degrees of freedom (df), *F* values (*F*) for the repeated measures of analysis of variance for stem water potential (Ψ), net photosynthesis (*A*), stomatal conductance (*gs*), transpiration rate (*E*), water use efficiency (*WUE*=[A/E]), intrinsic water use efficiency (*WUEi* = [*A/gs*]) and chlorophyll fluorescence (*Fv/Fm*) among three irrigation, two provenances and altitudes.

		F-values						
Source of variation	df	*A*	*gs*	*E*	*Ci/Ca*	*WUE*	*WUEi*	*Fv/Fm*
Between subjects								
Irrigation (I)	2	127.32***	101.58***	80.68***	13.40***	3.09*	14.51***	39.95***
Provenance (P)	1	7.43**	3.66	0.21	0.18	3.01	0.67	1.70
Altitude (A)	1	0.02	0.85	3.17	4.00	0.76	4.62*	1.05
Within subjects								
I*P	2	1.00	0.65	0.92	0.25	0.46	0.6	0.55
I*A	2	0.65	1.42	1.28	2.57	0.07	3.59*	0.64
P*A	1	0.00	2.79	0.04	6.05	0.02	10.59*	0.02
I*P*A	2	3.03*	3.71*	3.45*	2.99	0.03	1.51	0.17

Note: Significant level for repeated measures are given as corrected probabilities: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Table 5.4. Mean and standard errors of net photosynthesis (*A*), stomatal conductance (*gs*), transpiration rate (*E*).

Provenance	Altitude	Irrigation levels	*A* (µmol m^{-2} s^{-1})	*gs* (mol m^{-2} s^{-1})	*E* (mmol m^{-2} s^{-1})
Adapazari	High	3.70 cm/wk	7.28±0.16 a	0.09±0.00 ab	2.98±0.08 a
		1.25 cm/wk	6.28±0.24 bc	0.08±0.00 b	2.58±0.08 bcd
		0.00 cm/wk	5.31±0.19 d	0.07±0.00 c	2.38±0.08 cde
	Low	3.70 cm/wk	7.58±0.20 a	0.10±0.00 ab	3.04±0.08 ab
		1.25 cm/wk	6.54±0.23 b	0.08±0.00 ab	2.65±0.08 abc
		0.00 cm/wk	4.72±0.21 d	0.05±0.00 e	2.10±0.08 f
Karabuk	High	3.70 cm/wk	7.61±0.15 a	0.10±0.00 a	3.06±0.08 a
		1.25 cm/wk	6.84±0.24 ab	0.09±0.00 ab	2.74±0.08 abc
		0.00 cm/wk	5.38±0.20 d	0.06±0.00 cd	2.27±0.08 de
	Low	3.70 cm/wk	7.42±0.11 a	0.10±0.00 ab	2.87±0.08 ab
		1.25 cm/wk	6.78 ±0.22 ab	0.09±0.00 ab	2.64±0.08 abc
		0.00 cm/wk	5.57±0.19 cd	0.08±0.00 c	2.27±0.08 de

Note: Adapazari high altitude (>1400 m); Adapazari low altitude (1000-1309 m); Karabuk high altitude (1349-1472); Karabuk Low altitude (1000-1321m). Number followed by the same letter indicates no statistically significant differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence) under the interaction of irrigation x provenances x altitudes. ns: not significant.

There was a significant relationship between *A* and *gs* (R^2: 0.76, $P<0.0001$) when all provenance and altitude were combined. However, there was no statistical differences between altitude within each provenance (Fig. 5.5). There was a significant linear relationship between *A* and *gs* under the interaction of provenance and altitude (Fig. 5.5). The seedlings from AL and AH had higher correlation between *A* and *gs* (R^2: 0.82 and R^2: 0.79, respectively) compared to KL and KH (R^2: 0.70 and R^2: 0.73, respectively) (Fig. 5.5).

5.3.4.3. Transpiration rate (E)

Transpiration rate (*E*) was significantly affected ($P<0.05$) by I, T, IxT and IxPxA. Increasing irrigation increased the *E* when examining irrigation as a single factor. For IxPxA interaction, severely stressed seedlings from Adapazari lower altitudes had the lowest values (2.10 mmol m^{-2} s^{-1}) while well-watered trees from Karabuk higher altitudes had the highest values (3.06 mmol m^{-2} s^{-1}) (Table 5.4).

5.3.4.4. Ci/Ca, Water use efficiency [WUE (A/E)], and WUEi (A/gs)

Irrigation had a significant effect ($P<0.05$) on *Ci/Ca*, *WUE*, and *WUEi* with severely stressed trees having statistically lower values compared to medium and well-watered treatments for *Ci/Ca* and *WUE*, while severely stressed trees had higher values for *WUEi*. Altitudinal variation was significant ($P<0.05$) on *WUEi* that trees from higher altitudes had higher values (83 µmol/mol) compared to lower altitudes (81 µmol/mol). Under the interaction of PxA, trees from Adapazari lower altitudes had the highest *WUEi* values (86 µmol/mol) compared to other interactions while trees from Adapazari higher and Karabuk lower altitudes had the lowest values (79 and 81 µmol/mol, respectively).

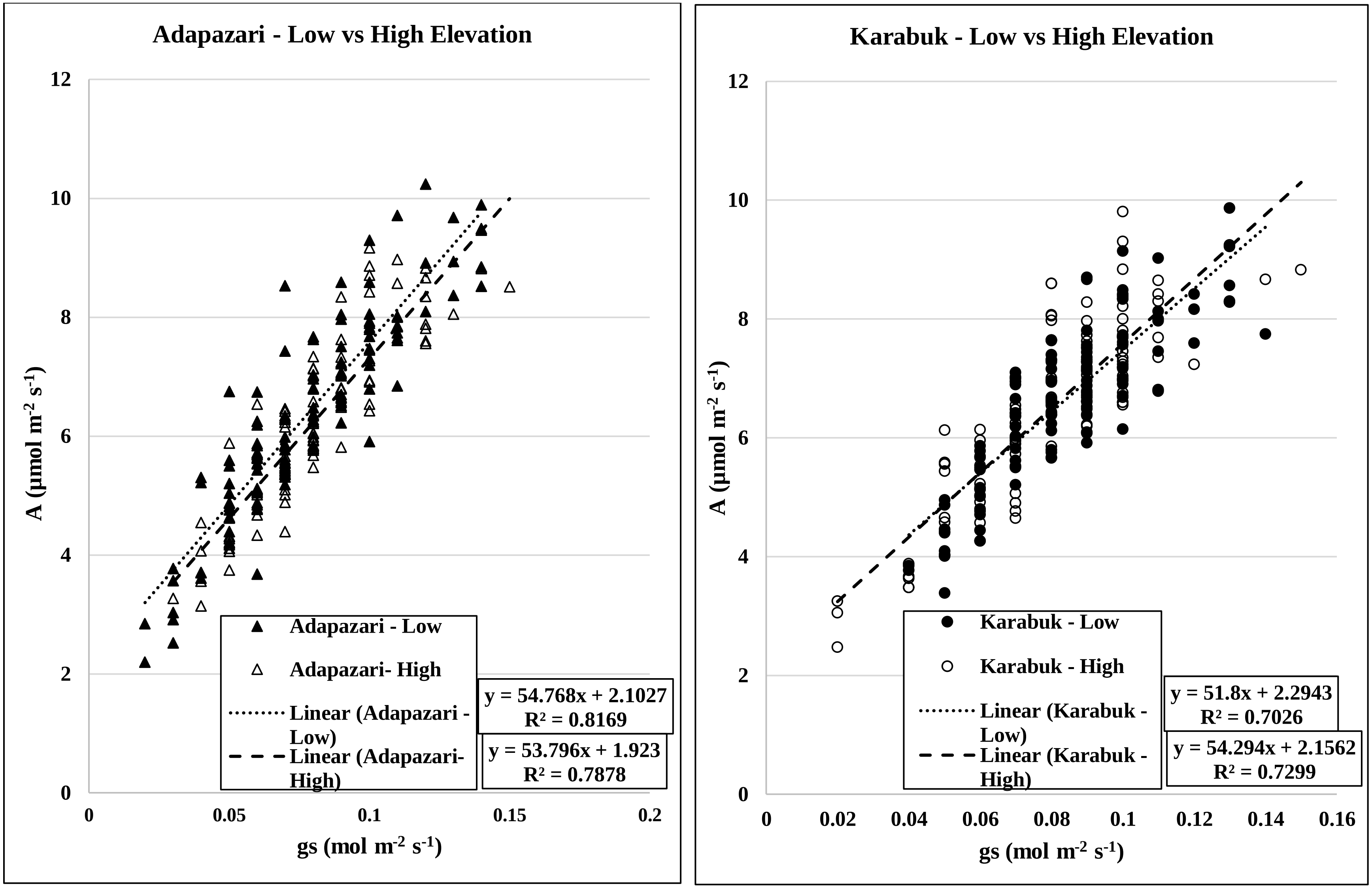


Figure 5.5. Relationship between *A* and *gs* in *Abies bornmuelleriana* seedlings from low and high altitudes of Adapazari and Karabuk provenances.

5.3.4.5. Chlorophyll fluorescence (Fv/Fm)

The *Fv/Fm* was significantly affected by irrigation (P<0.05) that well-watered seedlings had higher values (0.78) followed by medium irrigated seedlings (0.77) compared to non-watered seedlings (0.75) when examining irrigation as a single factor (Fig. 5.7). However, the altitude was not significant (P=0.30) (Fig. 5.6). The interaction of IxPxAxT was significant (P=0.003) on the *Fv/Fm* (Fig. 5.7) that the measurement of middle season result values was lower than the early and late season for all interactions except stressed trees from Adapazari higher altitudes. The severe-stressed trees from Karabuk lower altitudes had the lowest *Fv/Fm* values (0.70) in August 8, 2017 measurements.

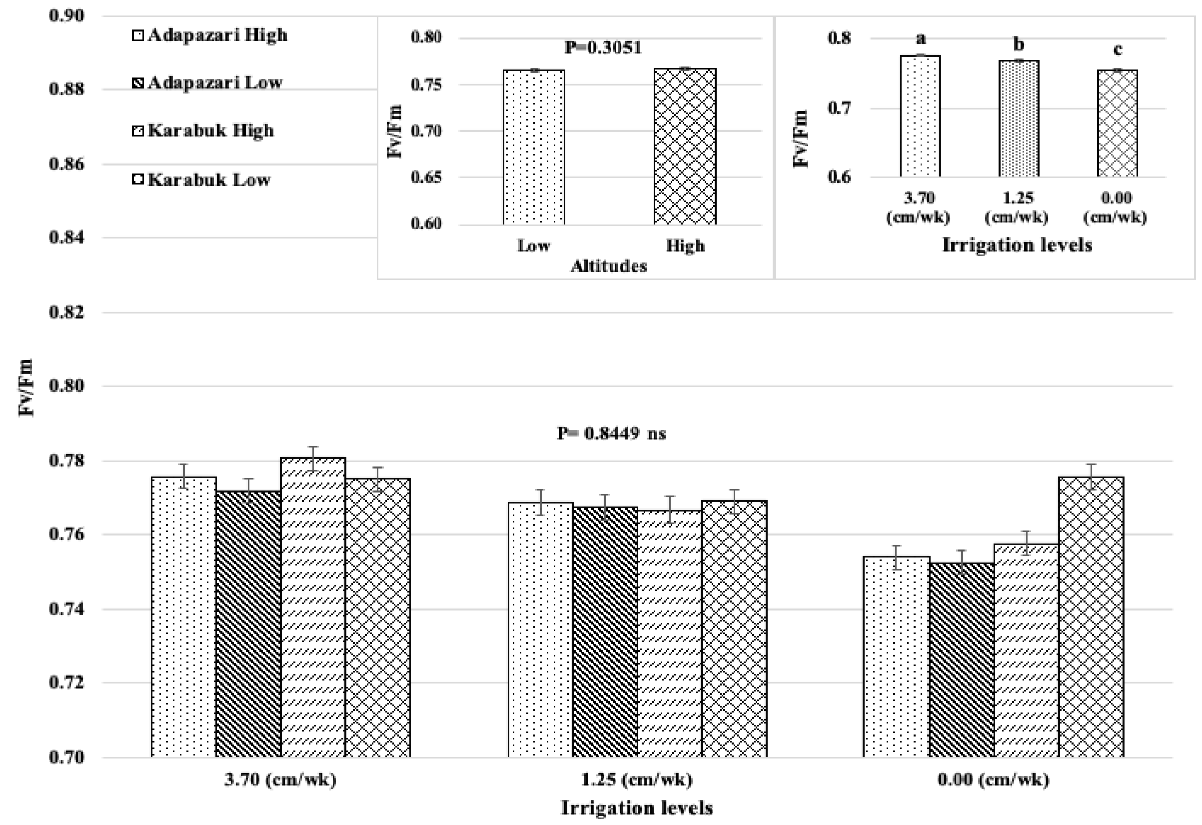


Figure 5.6. Mean and standard error of chlorophyll fluorescence (*Fv/Fm*) of Turkish fir under altitude, irrigation and provenance x altitude x irrigation interactions.

Note: Graphs followed by the same letter indicates no statistical differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence). ns: not significant.

5.3.4.6. Carbon isotope discrimination

Carbon isotope ratio [$\Delta^{13}C$] was not significant among tree provenance (P=0.43) and altitude (P=0.83), but well-watered seedlings had significantly lower values (21.16 ‰) than water stressed seedlings (21.49 and 21.50 ‰) (P=0.02) (Fig. 5.7). The interaction of each factors was not significant for $\Delta^{13}C$ (P>0.05) indicating that the rank of $\Delta^{13}C$ among factors did not change with water stress treatments (Fig. 5.8).

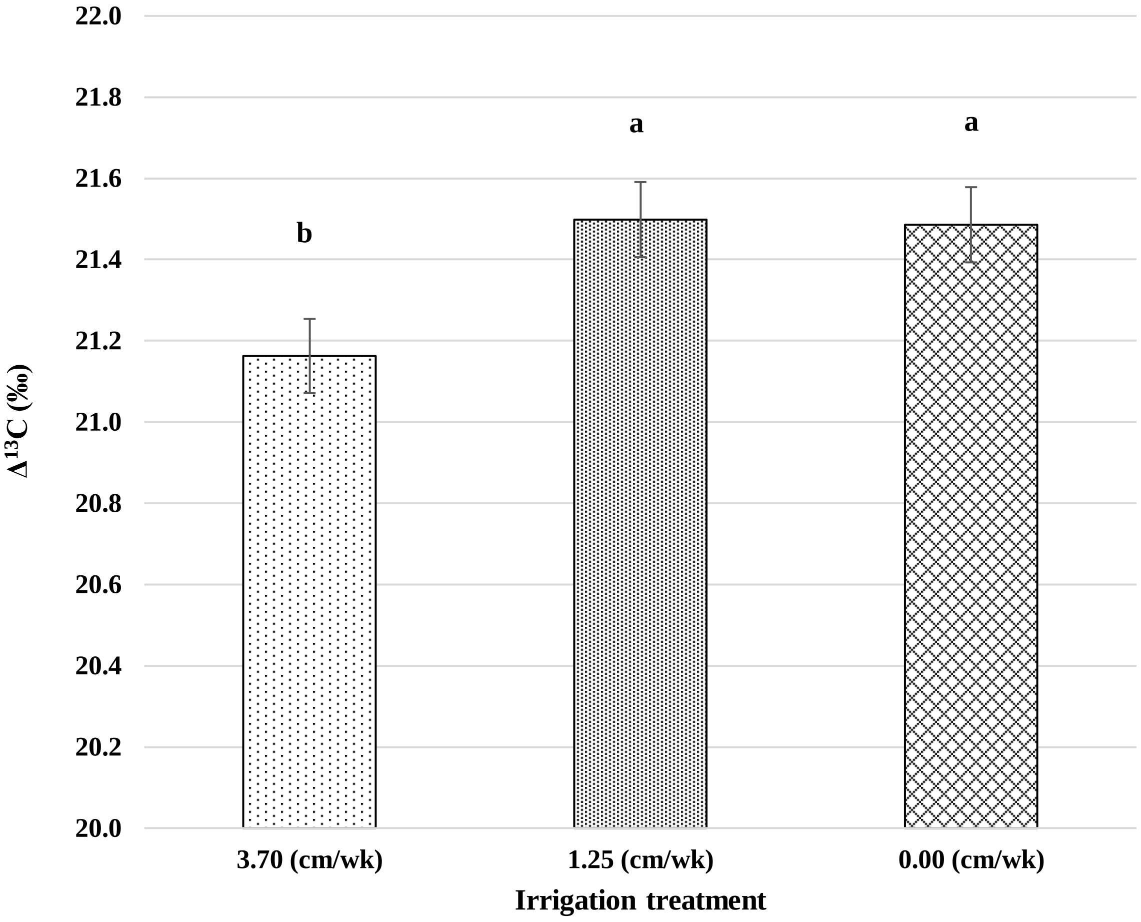


Figure 5.7. Mean and standard error of Turkish fir carbon isotope discrimination under three irrigation treatments.

Note: Graphs followed by the same letter indicates no statistical differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence.

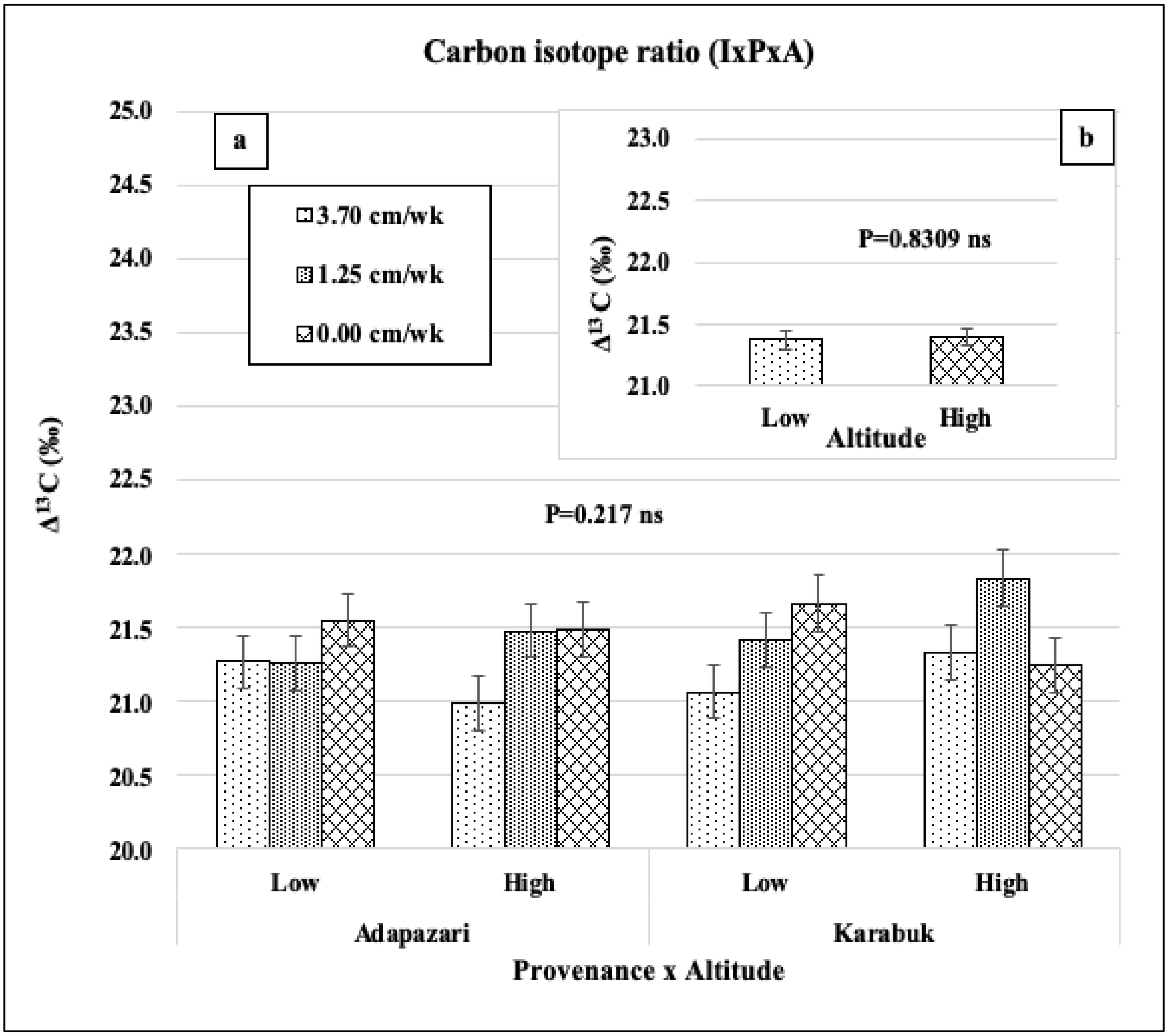


Figure 5.8. Mean and standard error of Turkish fir seedlings carbon isotope discrimination under the combination of irrigation x provenance x altitude (a), and altitude (b).

Note: Graphs followed by the same letter indicates no statistical differences (Tukey's Honestly-Significant-Difference Test with 0.95 confidence). ns means not significant.

5.4. DISCUSSION

5.4.1. Effect on growth response

Overall, irrigation level significantly affected RRCD while altitude had a significant effect on RRCD, RHG and stem volume index (d^2h). The positive effect of watering on RRCD growth observed in this study is not unexpected because it is established that enlargement of new tissues is mainly influenced by the water needed for shoot elongation and by ecological conditions throughout the season. Trees from higher altitudes showed a higher RRCD while there were no significant differences between provenances. Low irrigation and/or no irrigation caused elevated water stress on trees, reducing cell elongation and causing a net negative effect on growth (Klooster et al. 2010; Nzokou and Cregg 2010b). The irrigation levels and provenances did not differ on RHG which was expected due to most temperate trees including fir species form terminal buds during the previous year (Klooster et al. 2010; Nzokou and Cregg 2010). Seedlings from higher altitudes had not only higher RHG but also had nearly doubled in size (stem volume index; d^2h) compared to seedlings from lower altitudes. Trees from Adapazari higher altitudes had a greater in size in comparison to other interactions while trees from Adapazari lower altitudes had the lowest values.

5.4.2. Effect on plant physiology

Stem water potential was used as main indices of water stress in tested trees. Differences observed between irrigation treatment can be associated with water use by plants. The lower Ψ values are indeed due to reduced soil water and increased evapotranspiration linked to loss of vapor pressure that generates an increased deficit during midday (Williams and Araujo 2002). The stomatal conductance is regulated by water loss that leads to the depression of carbon assimilation because trees close their stomata during the day (Williams and Araujo 2002). In the study, there was a clear separation in Ψ between irrigation treatment. Trees under water stress, Adapazari at lower altitudes, had lowest Ψ values compared to other provenance x altitude interactions, where trees from Adapazari lower altitudes were more stressed from lack of irrigation. Also, trees from Adapazari lower and higher altitudes showed highest Ψ values under IxPxAxT interaction throughout the study period except August 31, 2017. Such seasonal changes have been observed from other *Abies* species (Tyree et al. 1978). Trees in general tend to keep a higher Ψ for continuous shoot elongation in the middle of growing season (Kulaç et al. 2012).

In our study, well-watered treatment had higher stem water potential (Ψ), net photosynthetic rates (*A*), stomatal conductance (*gs*), transpiration rate (*E*), water use efficiency (*WUE=A/E*) but lower intrinsic water use efficiency (*WUEi=A/gs*) in comparison to water-stressed seedlings among IxPxA interaction. The similar result are shown in Samuelson (2000) that net photosynthesis generally increases when there is available water in the soil (Samuelson 2000). Photosynthesis and stomatal conductance are strictly linked, the open stomata on leaf are to the efflux of water vapor when the influx of carbon dioxide. However, it is not surprising that the photosynthetic capacity is declined due to a strong reduction in CO_2 assimilation caused by stomatal closure (Zhang et al. 1997; Lawlor 2002; Ashraf 2003; Flexas et al. 2004) under imposed water stress which are observed in our study (Fig. 5.5). The balance among water loss and carbon gain regulates the degree of plants drought tolerance (Pinheiro et al. 2005). The accumulation of enzymes such as abscisic acids (ABA) in the needles occurs to induce stomatal closure that may cause a reduction in *gs* (Hopkins 1995).

Moreover, the two Turkish fir provenances did not differ for all gas exchange parameters except *A* where the Karabuk provenance showed higher *A* values compared to Adapazari provenance. Similar results were observed in pine species (Zhang et al. 1997; Zhan et al. 1996). The variation in phenology, gene, and needle morphology may compensate to keep gas exchange parameters at a constant value.

Similarly, as expected, *E* is impacted by the level of irrigation. Sever water stress causes a decline in transpiration and maintenance of cell turgor due to primary acclimation response mechanism to water stress (Hopkins 1995). We observed a decreasing trend in *WUE* in the middle of summer and increased towards the end of the season, similarly to findings reported not only for Fraser fir (Koc and Nzokou, 201X), but also *Quercus robur* (Welander and Ottosson 2000). However, intrinsic water use efficiency based on gas exchange measurements not only shows a relationship between *A* and *gs* but also showed a snapshot of plants' physiological activities at a given time. In our study, *WUEi* (*A/gs*) values increased with increased water stress due to a large reduction of stomatal closure compared to net photosynthesis.

In the study, chlorophyll fluorescence declined with exposed water stress. This is observed in Fraser fir (Kulaç et al. 2012). Due to reduction photosynthetic pigment in the result of low synthesis or faster breakdown of pigments caused by an escalation of oxidative stresses stimulated under water stress condition (Kulaç et al. 2012). Chlorophyll fluorescence is related to the changes in pigments concentration in tree needle that it is negatively affected by water stress.

Carbon isotope discrimination is used to provide a more precise and sensitive depiction of water stress due to ^{13}C playing a crucial role in tissue formation throughout growing period (Farquhar et al. 1989). However, there was no evidence of a clear relationship between drought tolerance and carbon isotope composition after one growing season for plants used in our study. In contrast, decreased irrigation increased Δ^{13}C. Similar results were observed in ponderosa pine (Zhang et al. 1997), Fraser fir (Kulaç et al. 2012) and other conifer species (Taylor et al. 2013). However, there was no observed difference due the altitudinal variation of the various provenances.

5.5. CONCLUSION

This study investigated the effects of the altitude at the seed collection site on early growth and physiology of Turkish fir. Water stress significantly affected relative root collar diameter while altitude significantly affected relative root color diameter, relative height growth, and stem volume index (d^2h). Plants originating from higher altitudes had greater growth compared to lower altitudes plants in Adapazari. Provenances significantly affected Ψ and *A* while other gas exchange parameter measured were not significant ($P>0.05$). Water stress reduced several physiological processes including Ψ, *A*, *gs*, *E*, *WUE* (*A/E*), *Fv/Fm*, and $\Delta^{13}C$ but there was generally no difference related to altitude of origin. In conclusion, our findings suggest that the altitude of origin affect early growth but not the tree physiology. Our study confirmed that water is a critical factor for growth and physiology regardless of the altitude of origin. However further work with wider differences in elevation between sourcing populations are needed for a better understanding of altitudinal effects on the growth and successful establishment of this species under various watering conditions. In addition, determination of additional physiological parameters (e.g. bud break) and biochemical and genetic markers would provide additional evidence to support or reject this hypothesis.

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CHAPTER 6

GENERAL CONCLUSSIONS

The research of this dissertation adds to the body of knowledge on stress-related plant morphology and physiology in conifer species, especially Eastern white pine (*Pinus strobus*), balsam fir (*Abies balsamae*), concolor fir (*Abies concolor*) and Turkish fir (*Abies bormuelleriana*) and is important for Christmas tree producers, and afforestation and plantation programs especially in the context of climate change.

Our results show that balsam and concolor fir species are better able to sustain their growth and physiological functions under water stress due to their ability to develop fine roots that could expand their water absorption capacity. Fir species had a higher net photosynthetic rate, stomatal conductance and transpiration rate, and a lower WUE compared to white pine. We conclude that white pine responds to water stress by quickly closing their stomata thus leading to a lower net photosynthesis and transpiration rate. This may have also impacted their higher observed WUE. Under water stress conditions, fir species not only keep higher total nitrogen concentration, but also had higher K^+ and lower Ca^{2+} concentrations compared to white pine seedlings. It is well known that increased K^+ concentration help maintain open stomata while increased Ca^{2+} concentration closes stomata. High Ca^{2+} concentrations as observed in white pine was therefore interpreted as an indication of stomatal closure in this species under stress. We concluded that two fir species were better able to adapt and maintain higher performances growth and physiology under water stress. However, white pine had higher drought tolerance because of their ability to withstand water stress by reducing their stomatal conductance, photosynthetic activities, and growth by minimizing water loss and increasing water uptake.

These results were also supported by our nutrient use physiology study where our results demonstrate that concolor fir had a greater capacity for holding acquired water compared to white pine, leading to better above-ground growth and S/R ratio. In addition, balsam and concolor fir exhibit higher nitrogen concentration compared to white pine due to their ability to maintain nutrient uptake with an expanding network of small roots under water stress conditions. However, white pine had higher assimilatory nutrient use efficiency, nutrient use efficiency, and root weight ratio, while a lower index of nitrogen availability compared to balsam and concolor fir, suggesting a better ability to tolerate water stress in these species. Increasing irrigation not only improved the resources use efficiency, but also improved other physiological characteristics.

Our studies of altitudinal differences in seed collection locations showed a significant effect on morphological traits with higher elevation showing better growth compared to lower elevations, this is attributed to early bud break and rapid development characteristic acquired in their natural range. In this study, well-watered plants had higher net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency, and chlorophyll fluorescence and lower intrinsic water use efficiency and carbon isotope discrimination compared to water-stressed specimens. However, provenance and altitudinal differences did not differ with most of these variables except stem water potential and net photosynthesis in provenances and intrinsic water use efficiency. The study suggests that altitude of the provenance does not significantly affect the early plant physiology under water stress, with water availability in the soil playing a more crucial role for growth and development. However, we recognize that other physiological parameters such as bud break could be more sensitive to the provenance’s altitude and should be considered before any definite conclusion is made of trends observed. For a better understanding of plant physiology in conifer species, future studies should focus on genetic research in addition to morphology.

In the perspective of future climate change scenarios, the future temperate forests climate will be characterized by more frequent heat and more widespread drought. Heat or increased temperature and drought will both challenge temperate conifer trees, such as pine and fir species.

Our results provide some information about plant physiology and morphology under water stress conditions that trees will be faced with in near future. The adaptation mechanisms of these species in drought condition will help us obtain better results of plantation and afforestation. The better selections of plant not only increase forest areas or prevent from deforestation, but also it helps to emit and sink carbon from the atmosphere, which is the main driver of global warming. The current studies will need support with provenance and genetic studies that will reveal different aspects of how these species respond and adapt to water stress. Early attempts at predicting phenotypes from genotypes suggest that genetic tools may be able to create a better selection of plant species in the future.