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Physiological variation of Garry oak (Quercus garryana) seedlings to drought stress

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PHYSIOLOGICAL VARIATION OF GARRY OAK (*QUERCUS GARRYANA*)

SEEDLINGS TO DROUGHT STRESS

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Master of Science

Biology

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by

Matthew A Merz

June 2015

CENTRAL WASHINGTON UNIVERSITY

Graduate Studies

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ABSTRACT

PHYSIOLOGICAL VARIATION OF GARRY OAK (*QUERCUS GARRYANA*)

SEEDLINGS TO DROUGHT STRESS

by

Matthew A Merz

June 2015

The purpose of this study was to determine physiological differences in drought response among Garry oak (*Quercus garryana* Douglas ex Hook. [Fagaceae]) seedlings that grow in distinct populations in Washington state, in order to aid in restoration efforts by informing growers of the potential differences between the acorn collection sites. Acorns from six Washington populations east of the Cascades, as well as one population from Whidbey Island, were collected and grown in containers under controlled conditions. The plants were assessed with and without moderate and severe drought stress induced by withholding water. The most extreme differences in photosynthetic characteristics for non-stressed seedlings from different populations were in stomatal conductance rates, which in some cases differed between populations by 2 fold or more. Differences in response to drought occurred across populations after 14 days of withholding water (Fig. 4). Seedlings from the northernmost population of Garry oak on the east side of the Cascades (Swauk Creek) were most susceptible to lowered photosynthetic rates induced by drought stress, while an island population from the west

side of the cascades (Oak Harbor) showed the least drought-induced reduction of

assimilation. Differences among populations were also observed in the occurrence of

drought induced embolisms, and in changes in leaf water potential, relative water content,

and production of the osmolyte proline in response to drought. This study shows that

seedlings from different populations of Garry oak have distinct physiological responses

to drought that may lead to changes in survivorship for seedlings used in reforestation

efforts for Garry oak savannas as they are exposed to drought stress in the field.

AKNOWLEDGEMENTS

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CHAPTER I

GENERAL INTRODUCTION

Introduction

Growth and productivity in plants is facilitated by photosynthetic reactions that convert light energy into chemical energy. When growth conditions, including temperature, light, water, and nutrient availability, are ideal, plants achieve their highest growth and reproductive potential through maximum efficiency of their photosynthetic reactions. However, in the multitude of environments in which plants occur throughout the globe, few growth habitats exist in which ideal growth conditions occur for much of the time. Terrestrial plants encounter a variety of environmental stresses which have long been known to limit plant productivity worldwide (Boyer 1982). The most common forms of abiotic stress for plants in temperate climates include high or low temperature, solar radiation extremes, and lack of water availability (e.g. Larcher 1995). Because of the importance of effects of environmental stress on plant productivity, much current plant research has addressed the mechanisms by which plants respond to one or more environmental stresses (Mittler 2006). Changes in global climate have the potential to exacerbate environmental stresses for plants in regions where longer, warmer and more frequent droughts will occur in many regions of the world (Parry 2007) and such climatic modifications have been associated with regional tree mortality events in southwest United Sates (Breshears et al. 2009). Increasing our understanding of how plants in both natural and agronomic environments respond to abiotic stress is of utmost importance in facilitating our response to climate change.

Plant Responses to the Environment

Drought stress, is a condition in which plants have less available water than they need for maximum growth. Plant responses to drought are complicated because plants must continually replace the water they lose. Typically plant roots absorb water from the soil, where it is then transported through the roots and through the vasculature of the stem to the leaves. Water is ultimately evaporated from leaves into the environment through regulated pores in the leaves called stomates through a physiological process called transpiration. Transpiration serves several functions in plants including movement of nutrients obtained from the soil into regions of the plant where they are needed for growth. Evaporation of water from leaves through transpiration is a cooling process and therefore the process of transpiration serves to regulate leaf temperatures and dissipate heat that they acquire through absorption of solar radiation throughout the day. Transpirational water loss through stomates is a necessary consequence for leaves that must open stomates to provide a pathway for carbon dioxide uptake for photosynthesis.

Carbon dioxide is a gas that is present in the air at a relatively low concentration (0.04%) as compared to nitrogen (78%) and oxygen (21%) which are the main components of our atmosphere. Plants require carbon dioxide for photosynthesis as they use light energy to convert the carbon dioxide into sugars that are required for basic plant metabolism. Because above-ground regions of plants are effectively covered in a protective, hydrophobic layer called the cuticle, stomates are the only pathway for entrance of carbon dioxide into leaves for photosynthesis. Opening stomates for carbon dioxide, however, presents the necessary cost of water loss through the same opening.

The amount of water loss is increased when evaporation rates are the highest, which occurs when the temperature is high, or the vapor pressure of the air surrounding the leaf is low. When the water demands of a plant are in excess of the available water, there are several different mechanisms plants employ that although they may limit photosynthetic productivity, can be used in order to avoid damage or death as a result of droughted conditions. One common mechanism for drought-avoidance is to physically limit the pore size through which water evaporates from the leaves by closing stomatal pores which reduces water loss at the cost of decreased photosynthetic rates, thereby lowering plant productivity under times of limited water availability (Mediavilla and Escudero 2004). The pores in the leaves of plants are flanked on each side by a pair of specialized cells called guard cells which can open or close the pore by increasing or decreasing their intercellular pressure. Limiting the flux of water escaping the stomata also limits the flux of CO_2 inward that can be used for photosynthesis. This is known as a stomatal limitation to photosynthesis. Many factors can affect stomatal closure including, but not limited to, vapor pressure deficit of the atmosphere, annual rainfall, and soil texture and structure (e.g. Larcher 1995).

Photosynthesis can also be limited by non-stomatal mechanisms as a result of drought. If the amount of available water is less than that needed for normal growth, but the leaves are still receiving high levels of solar energy from the sun, the plant can be damaged by the excess energy (Bjorkman 1981). The wavelength range of the photosynthetically active portion of radiation that Earth receives from the sun is 400 to 700 nm. The intensity of light that is available for photosynthesis is often measured as

the number of photons that reach the Earth's surface, per unit area, per unit time. Photons are elementary particles of light energy. The amount of light that plants encounter in the natural light environment can vary greatly with time and location. For example, the quantity of light that reaches the shady understory of a tree canopy can be as low as 0.05 - 25% of the amount that hits the canopy of the tree.

Photosynthesis takes place in organelles called chloroplasts. Photosystem I (PSI) and Photosystem II (PSII) are the two reaction centers within the chloroplasts. Both of these reaction centers convert light energy to chemical energy that is subsequently used to reduce, or "fix" atmospheric CO_2 through a complex metabolic pathway called the photosynthetic carbon reduction pathway, or the "C_3" pathway. When stomates are closed in order to prevent loss under drought conditions, there is a very limited amount of CO_2 available for the photosynthetic carbon reduction cycle. The problem is exacerbated at low or high temperatures that decrease rates of enzymatic reactions. Under such conditions there is a reduced capacity for photosynthetic reaction centers to quench excitation energy from light absorption because without CO_2 or enzymatic activity, there is essentially no outlet for absorbed light energy. Under these conditions, the reaction center PSII is susceptible to light-induced protein degradation that lowers the photosynthetic capacity of plants and is called photoinhibition (e.g. Cornic 1994).

Plants have the capacity to protect themselves from photoinhibition that might potentially occur under drought or other environmentally stressful conditions, such as low or high temperatures, through reactions that dissipate excess excitation energy as lower wavelength energy or, more frequently, as heat (e.g. Demmig-Adams and Adams 1992).

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These energy dissipation pathways are a way of down regulating the photosystem, which decreases the efficiency of photosynthesis but prevents photoinhibitory damage to PSII. If light energy exceeds the capacity that plants have to protect themselves from photoinhibition, protein synthesis is required in order to rebuild damaged PSII. To accomplish this costs the plant valuable stored energy. Whether the reaction centers are being down regulated or destroyed, the overall photosynthetic capacity of the plant is reduced through these processes.

Plants also respond to the environment through biochemical changes that determine maximum carbon assimilation rates, cellular respiration rates, and nutrient use efficiencies. Plants also respond through anatomical changes such as changes in leaf thickness, surface area, the number of stomates per unit leaf area, and through changes in growth such as modification of the ratio of root to shoot biomass (e.g. Larcher 1995). Thicker leaves often contain a higher concentration of the important photosynthetic pigment, chlorophyll, and are therefore capable of higher rates of photosynthesis. Likewise some plants with thinner leaves contain less chlorophyll and have lower photosynthetic rates. Differences in leaf thickness and chlorophyll concentration can often be correlated with the light environment of the plant, with the thicker leaves and higher photosynthetic capacity occurring in high light, and thinner leaves with lower photosynthetic capacity occurring in low light. These differences can even be seen on the same plant, when comparing sun leaves from the brightly lit top of the canopy to shade leaves from the shaded understory (Boardman 1977).

Plant Water Relations

In order to quantify the force by which plants pull water from the soil, researchers often employ references to the concept of plant water potential, or Ψ_w. Water potential is the chemical free energy for water movement (or driving force of water) between systems. A major component of the water potential that determines the driving force for water movement is the solute potential. Much like the flow of water down a pressure or gravity gradient, free water will move away from an area of low solute concentration to areas of increasing solute concentration. Some plants, respond to drought through adjustment of their cellular water potential by the production of osmolytes or solutes that decrease the cellular Ψ_w and increase the force by which water will move into a cell (Karamanos 2008). One such osmolyte is the amino acid, proline. Plants that produce proline in response to drought can make use of soil water that was previously unavailable to them (Ábrahám et al. 2010).

Water movement in plants is facilitated by vascular tissue which is comprised of two basic cell types, xylem and phloem. Xylem transports water up from the roots of the plant, through the stems, and into the leaves. Phloem transports the sugars synthesized during photosynthesis away from the leaves. In woody plants, such as trees, the bulk of the above-ground biomass is made up of xylem cells in the trunk of the tree that transport the large volume of water required for transpiration in a massive crown of leaves. Stem hydraulic conductivity is the measure of the flux of water (conductance) through the xylem. In ring porous angiosperm trees, water is transported primarily in the outer-most rings of xylem in continuous connected elements called vessels (e.g. Evert 2006).

A problem that can arise during the transport of water is cavitation, which is the formation of embolisms, or air bubbles that form a break in the continuous water column that normally occurs from the roots to the stem to the leaves through a tree. Embolisms form when tension on the water column causes air that is dissolved in the xylem sap comes out of solution. Many plants experience embolisms on a daily basis that occur due to temperature and pressure change (Tyree and Sperry 1989). Minor embolisms can be repaired at night when transpiration rates are low or as the path of water is re-routed around them. However if there is very limited water in the soil and the gradient in water potential between the soil and the leaves is particularly high, or if freezing of the xylem sap occurs, embolisms can form more readily and recovery can become much more difficult for the plant to deal with. Indeed if cavitation is extensive the plant may be doomed and cannot recover without the production of new wood and a new water column in the next season (Tyree and Sperry 1989).

Arid environments typically receive anywhere between zero and 30 cm of annual precipitation, and receive more light energy than the equator during the growing seasons (Renard et al. 1993). Plants that live in arid or semi-arid environments are often affected by drought and are often at the physical limits of what they can tolerate (Tognetti et al. 1998). This means that an increase in drought in a semi-arid environment, even if it is relatively small, can tip the balance against the plants causing a decline in the population. One such decline occurred in a pinyon pine (*Pinus edulis*) population near Los Alamos, NM in 2000-2003 (Breshears et al. 2009). In this example, the reduction of annual rainfall resulted in the loss of more than 90% of the established pinyon pines in a single

year. One of the major concerns for areas such as these is the reduction in annual rainfall as a result of global climate change, and its impacts on the local plant life (Hanson and Weltzin 2000, Leung et al. 2004, Allen et al. 2010). Understanding the ways that drought-tolerant plants deal with drought and the survivable extreme limits of drought stress for those plants are important in a changing environment.

Garry Oak Savannah Ecosystems in Washington State

In the Pacific Northwest oak woodlands and savanna inhabited by Garry oak (*Quercus garryana*) are semi-arid environments. Garry oak, also known as known as Oregon white oak, is a broadleaf deciduous tree in the family Fagaceae and is the only oak species native to Washington State. The difference between the Garry oak woodlands versus savannas mostly pertains to the patterns in which the trees grow. Savannas generally have a very broken mosaic pattern of canopy cover with patches of open canopy where full sunlight reaches the understory. Woodlands are more densely populated with trees and the canopy intercepts most of the sunlight. Garry oak is often interspersed with woodlands of Douglas-fir (*Pseudotsuga menzisii*), lodgepole pine (*Pinus contorta*), and ponderosa pine (*Pinus ponderosa*). These woodlands and savannas are often organized at a large scale into a mosaic of communities across the landscape, with intermingling boundaries of combined communities (Dunwiddie et al. 2011). The patchy distribution of trees in oak savannas allows high amounts of sunlight to reach the understory, which makes it possible for plant, and animal species to occur that would not be present in the woodland distribution.

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Garry oak savannas in Washington State have been in decline for the last 150 years or so, with drastic declines seen in the last 50 years. It is estimated that only 5% of the historical Garry oak savannas exist today (Fuchs 2001). Garry oak savannas are more diverse than Douglas fir woodlands and are an important ecosystem in the Pacific Northwest, providing habitat for thousands of species of plants, animals, and other associated organisms. Some of these species are unique to the oak savannas and, as a result of the reduction in habitat, have become threatened in Washington State. One such organism is the western gray squirrel (*Sciurus griseus*) which use the trees as nesting sites, and depends on the acorns as an important food source (Dunwiddie et al. 2011). For these reasons there has been increasing concern in not only protecting what remains, but restoring some of the historical populations. This involves replanting seedlings of Garry oak in the reforestation locations. However, little is known about how Garry oak seedlings will respond to drought after outplanting or whether there is a threshold of drought for these trees that limits their distribution.

Growth, Physiological and Distribution Characteristics

Garry oak is a drought tolerant, shade intolerant oak species. Garry oaks can withstand drought extremes rivaled only by a few other oak species and they accomplish this in a number of ways. Garry oaks grow very slowly which makes them poor competitors and sensitive to encroaching competitive trees such as Douglas fir (Gould et al. 2011). A slow growth rate is particularly stressful to ring porous trees such as Garry oak, as they can only transport the bulk of their required water through a relatively small amount of xylem in the outer two rings of sapwood. This is a challenge for the trees

because they have to balance their growth rate to use a minimum amount of water so that they do not run out of water in the summer months, but at the same time they must carry out enough growth in the season to generate adequate new functional xylem to transport water during next growth season (Gould et al. 2011). Garry oak also exhibits different growth forms depending on the available water of their habitat, with smaller shrubby growth forms around five meters tall in dry areas, and large tall tree forms reaching 30 meters in wetter areas (Devine et al. 2013).

Another adaptation to drought achieved by Garry oak trees is the ability to maximize use of stored water. They do this by charging their sapwood with water in spring which is their primary growing season, and in part, using that reserve water through the dry season. They then recharge again in the fall when the rains return (Gould et al. 2011). This is a tactic not uncommon among large woody trees, and it becomes more important with increasing tree size. The larger the tree, the more reliant it is on stored xylem water (Phillips et al. 2003). A characteristic of Garry oak that does not fit with most trees is the fact that as the trees grow, their leaf area to sapwood area ratio increases. This means that there is more evaporative leaf surface area in a larger oak with less sapwood to move the water to the leaves. Most trees decrease this ratio to compensate for the evaporative demand. The reason that large Garry oak trees don't lose their drought tolerance as they get older is partially that they have lower water flux and are more efficient with water than when they were smaller (Phillips et al. 2003). Lower transpiration rates and higher water use efficiency, suggest that these larger trees have a decreased stomatal conductance compared to when they were young, supporting the idea

that photosynthesis is limited by stomata such that growth rates are slower, but less water is lost.

Large early wood vessels in oaks are the most vulnerable to drought or freezing-induced embolisms in their xylem (Sperry and Sullivan 1992). Smaller diameter vessels can better recover from embolisms (Logullo and Salleo 1993). White oaks such as Garry oak which are a sub-genera of oaks (*Lepidobalanus*) sensu Camus, generally have smaller vessels than red and evergreen oaks which belong to the sub-genera (*Erythrobalanus*) sensu Camus (Bonner and Vozzo 1987, Cavender-Bares and Holbrook 2001). This means that the smaller vessel size of white oaks as compared to other oaks is a sort of tradeoff, trading a lower flux of water through the stems to the leaves for less risk of permanent damage or death due to drought-induced embolisms. Although hydraulic conductivity in response to drought has been determined for many oaks including some white oaks (e.g. Cavender-Bares and Holbrook 2001) stem hydraulic conductivity characteristics for Garry oak have not previously been investigated.

Garry oak trees are very shade intolerant and rely on an open, broken canopy characteristic of savannas (Gould et al. 2008). In general, oaks do most of their growing in the spring, and therefore need the maximum light levels available during this time (Dougherty et al. 1979). When grown in shade, the thickness of the leaf as well as the chlorophyll content tend to decrease (Aranda et al. 2005). The biomass and photosynthetic machinery produced by both sun and shade leaves comes at a cost of energy, and both conditions have potential downsides. If more light is available, the

energy can be wasted, whereas if light is limited, the plant must be efficient with what light it has in order to maintain adequate photosynthesis.

Of the many oaks species in the world, the more drought tolerant species tend to have better control and faster response time for their stomates, and often have less non-stomatal limitations to carbon assimilation (Dickson and Tomlinson 1996). Bur oak (*Quercus macrocarpa*) like Garry oak is a white oak and has been shown to have a two to seven-fold increase in leaf hydraulic conductance in response to light increase, compared to a 1.5 fold enhancement experienced by other oak species such as the red oak (*Quercus rubra*) (Voicu et al. 2008). This further illustrates the importance of light to oaks as well as the efficiency with which drought tolerant species use water.

Regeneration Patterns for Garry Oak Stands

All oaks are monoecious, having separate male and female flowers both occurring on the same tree. Oaks are wind pollinated, and are self-incompatible (Fuchs 2001). Because of this, all viable acorns produced by Garry oak occur from a cross pollination event between two different trees. Garry oak is a recalcitrant oak species, which means that there is no embryo dormancy in the current year's acorns. Once dropped, the acorns maintain a high rate of cellular respiration, require a high amount of moisture retention to remain viable, and germinate almost immediately (Devine et al. 2010). The acorns are often buried by small mammals, which greatly increase their chances of survival. It is estimated that more than 65% of acorns buried by animals germinate and survive their first year (Fuchs et al. 2000). Un-germinated acorns of Garry oak do not last more than one year in nature, but have been shown to stay viable for up to two or three years if kept

at low moisture content in cold dark storage at about 4°C (Devine et al. 2010). Acorn

production shows a trend with annual precipitation, with years of high production

typically following late winter (February) precipitation followed by early summer (June)

precipitation. Interestingly the early summer precipitation also correlates with no acorn

production in the following year (Peter and Harrington 2009). Typically following a year

of heavy acorn production, there is a year of little to no acorn production. When

collecting acorns for growing and reforestation efforts, it has been suggested that the

collection site be as close to the target planting site as possible (Devine et al. 2013).

Acorns may be disperse naturally by birds and other animals up to 300 meters away from

the parent tree. If acorns are to be collected and moved further than that, one general rule

suggested is to collect from an area differing by no more than 2 cm annual precipitation

or 1°C in mean temperature for the warmest part of the year (Devine et al. 2013).

Garry oak savanna structure maintenance is dependent on recruitment of new

trees into the stand. Seedling establishment is an important factor in the stand

development of many tree species, especially in areas that are disturbed, or that are

environmentally stressful to the plants. Garry oak seedlings are susceptible to

environmental stress in the form of drought, high and low light intensity, herbivory, and,

at least historically, disturbance by fire. It is estimated that in dry rocky reforestation

sites, drought kills an estimated 59-78% of new seedlings (Fuchs et al. 2000). Mature

trees are quite adapted to fire, but the seedlings are very vulnerable. Regardless of this,

the benefits of the removal of competitive species by fire far outweigh the loss of

seedling recruitment (Adams and Rieske 2003). Many oak species including Garry oak

employ phytochemical responses to herbivory in the form of changes in foliar carbohydrates, and tannin concentrations. As a result Garry oak do not have many large herbivore species that regularly eat the leaves, and the insect species that do are not usually damaging enough to result in mortality (Devine et al. 2013).

Most oak seedlings perform better in full sun than in shade, exhibiting higher photosynthetic rates and water use efficiencies when not shaded (Rebbeck et al. 2012, Aranda et al. 2007). Garry oak seedlings are usually most abundant under the partially shaded canopy of other oak and non-oak trees, and are least abundant in the open patches of a savanna (Michalak 2011). This would suggest that typically the seedlings of Garry oak are operating at a lower photosynthetic rate and may have a lower water use efficiency than if they were located in the full exposure of the open patches. One study showed that in areas with more available water, competition was the main inhibitor of oak seedling establishment, and the fewer plants around the oak the higher the chance of survival. In drier areas however the surrounding vegetation becomes more important because it acts as a boundary layer which resists mixing of air, increasing the relative humidity around the seedlings, reducing the vapor pressure deficit in the immediate area (Muhamed et al. 2013). It has also been shown that many oak species can survive in a light intensity that is as little as 5% of full sun, however they require at least 20% and up to 80% depending on the species to maintain consistent stem growth (Brose 2011).

Garry oak forms mycorrhizal relationships with symbiotic ectomycorrhizal fungi, particularly with those in the genus *Tuber* and *Laccaria* (Southworth et al. 2009). These associations begin to form immediately after germination and are a benefit to the

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seedlings in several ways. Mycorrhizae aid plants in absorption of water and nutrients

acting as extensions to the root systems, allowing the plant access to water and nutrients

that would otherwise be unavailable. There are also some conspecific interactions

between trees associated with mycorrhizal fungi. It has been shown that with some oak

species, seedlings with mycorrhizal associations grow bigger and break bud earlier than

seedlings without them (Dickie et al. 2007). Container-grown oak seedlings used in

replanting efforts often form mycorrhizal relationships in the container, but lack the same

types and richness of mycorrhizal species of those that germinate in the field (Southworth

et al. 2009). The spores and mycelium of the associated fungal species often persist in

the soil after the host species has been removed or pushed out by an invader. For this

reason restoration efforts are often more successful in areas where the target plant

previously existed (Schaefer 2011).

The purpose of this investigation is to determine the physiological differences, if

any between populations of Garry oak in Washington State in order to assess differences

in drought-relevant physiological characteristics, such as maximum photosynthetic rates,

and stomatal conductance to water vapor for unstressed as well as drought-stressed

seedlings. Growers of seedlings to be used in restoration efforts often gather seeds from

several different sites in order to have a variety of ecotypes. Information from this study

will aid in restoration efforts by providing information to growers about potential

differences in drought tolerance for Garry oak acorns collected from different seed

sources. This research will also add to the pool of knowledge about drought stress

physiology for seedlings from deciduous trees. As drought becomes more of a concern

due to climate change in places that are already arid climates, this information will be useful in making management decisions about this important ecosystem in the Pacific Northwest.

CHAPTER II

JOURNAL ARTICLE

Physiological Response of Garry oak (*Quercus garryana*) Seedlings to Drought

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Running head: Response of Garry oak seedlings to drought

Abstract: The purpose of this study was to determine physiological differences in drought response among Garry oak (*Quercus garryana* Douglas ex Hook. [Fagaceae]) seedlings that grow in distinct populations in Washington state, in order to aid in restoration efforts by informing growers of the potential differences between the acorn collection sites. Acorns from six Washington populations east of the Cascades, as well as one population from Whidbey Island, were collected and grown in containers under controlled conditions. The plants were assessed with and without moderate and severe

drought stress induced by withholding water. The most extreme differences in photosynthetic characteristics for non-stressed seedlings from different populations were in stomatal conductance rates, which in some cases differed between populations by 2 fold or more. Differences in response to drought occurred across populations after 14 days of withholding water (Fig 4). Seedlings from the northernmost population of Garry oak on the east side of the Cascades (Swauk Creek) were most susceptible to lowered photosynthetic rates induced by drought stress, while an island population from the west side of the cascades (Oak Harbor) showed the least drought-induced reduction of assimilation. Differences among populations were also observed in the occurrence of drought induced embolisms, and in changes in leaf water potential, relative water content, and production of the osmolyte proline in response to drought. This study shows that seedlings from different populations of Garry oak have distinct physiological responses to drought that may lead to changes in survivorship for seedlings used in reforestation efforts for Garry oak savannas as they are exposed to drought stress in the field.

Keywords: environmental stress, water stress, Oregon white oak, soil water content, oak restoration, cavitation

Introduction

Garry oak (*Quercus garryana* Douglas ex Hook. [Fagaceae]) savannas have a typical savanna appearance, with loose clusters of trees intermingled with areas of open canopy (Dunwiddie et al. 2011). There are more species associated with Garry oak savannas than there are with Douglas fir woodlands and there are some species that are unique to Garry oak habitat (Thysell and Carey 2001). Among the numerous important species associated with Garry oak savannas are the golden paintbrush (*Castilleja levisecta*), Lewis' Woodpecker (*Melanerpes lewisii*), and the western gray squirrel (*Sciurus griseus*). The habitat for western gray squirrels is becoming seriously threatened in Washington State (Dunwiddie et al. 2011), and it is estimated that only 5% of the historical oak savannas remain today (Fuchs 2001). For these reasons oak savannas are important from an ecological standpoint and there has been increasing interest in restoring and reestablishing historical distributions.

Garry oak woodlands and savannas in Washington state exist on both the east and west side of the Cascade Mountain range. The west populations are primarily in the Puget Sound Area (Thysell and Carey 2001). Much of the western Garry oak communities have been lost and the land converted into agricultural or developed land (Dunwiddie et al. 2011). Historically the oak savannas oscillated with the Douglas fir woodlands, with fire being the main mechanism for maintenance (McDadi and Hebda 2008). Garry oak stands can take hundreds of years to develop into mature savannas. Cores of established oaks indicate that the primary recruitment of current established oak communities occurred between 1850 and 1890, and there has been very little further

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establishment since fire suppression began in the 1950's (Gilligan and Muir 2011). The structural development of the remaining oak stands is very different from what was experienced from 1700 to 1800, and perhaps further back than that (Sensenig 2013).

Garry oak populations on the east side of the Cascade Range in Washington state are limited to a few scattered pockets from Swauk Creek (Kittitas Co.) south to the Columbia River and into Oregon. These pockets are possibly all genetically similar enough to be considered the same distinct population, however no genetic work has been done on these specific groups of trees (Devine et al. 2013). When using isozyme variation to compare Garry oak populations, there is a trend of less genetic relatedness between populations as you travel north along their range (Ritland et al. 2005). In some cases populations just a few hundred kilometers from each other in Washington State are less related to each other than they are to populations in southern California (Ritland et al. 2005).

Oaks from co-occurring species in the same location and of the same species from different locations have been shown to respond differently to resource limitations. Co-occurring oak species in the Eastern US have different strategies for resource use such that one species, the chestnut oak (*Quercus prinus*) is more conservative with its use of water and nitrogen while another, the eastern black oak (*Quercus velutina*) maintains higher photosynthetic and nitrogen use values at the cost of extra water use (Renninger et al. 2014). Populations of Mediterranean oaks from drier climates survive drought better than those of the same species from wetter climates, suggesting a difference in ecotype between the two climates (Andivia et al. 2012). For other oaks, there are a high degree of

biochemical differences within species over an elevation gradient, including differences in the occurrence of dehydrin proteins that help plants tolerate drought stress (Vornam et al. 2011). Garry oak savanna microclimates are highly variable and have different temperature extremes, annual precipitation, and elevations (Gilligan and Muir 2011). The scattered populations of Garry oak on the east side of the Cascade Range may be separated enough and experiencing different enough microclimates to have different adaptations to drought stress. This is further illustrated in the recommendation that acorn collection for reforestation efforts should take place as near to the target planting site as possible (Devine et al. 2013).

Garry oak savanna structure maintenance is in part dependent on recruitment of new trees into the stand. Seedling establishment is an important and difficult event for many tree species. It is especially difficult in areas that are disturbed, or that are environmentally stressful to the plants. Drought kills an estimated 59-78% of new seedlings located in dry rocky sites in reforestation efforts in British Columbia (Fuchs et al. 2000). Because of the interest in restoration of Garry oak savannas there has been some work done on how to optimize first year survival of the seedlings post planting. It has been shown that after planting, the seedlings have much higher survival rates if they are irrigated for their first year (Devine et al. 2007) and that seedlings with more, shallow fibrous roots survive better after transplanting in the field (Gould and Harrington 2007). This is most likely because the fibrous root systems of the seedlings can more rapidly adapt to changes in the environment such as drought, and can more efficiently utilize shallow water sources. It has also been shown that tree shelters placed around the

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seedlings which reduces the amount of water lost to evaporation, improve their chances of surviving their first year (Kjelgren et al. 1994, Devine et al. 2007). Tree shelters also cause the seedlings to grow tall quicker which puts them out of the reach of browsers at an earlier age (Devine et al. 2007, Wells 2010). Browsers, however, do not pose significant threat to the seedlings of Garry oak as they are adapted to loss of the shoot apex and can readily re-sprout from below ground provided they have adequate root mass (Devine et al. 2013).

Garry oak seedlings have four major hurdles to overcome in order to survive their first year: 1) The acorns must be buried and have the adequate moisture for quick germination (Fuchs et al. 2000). This is usually accomplished in the environment with the aid of small mammals such as the western gray squirrel, or in nursery operations by germinating the acorns in containers. 2) The seedlings must avoid being damaged or destroyed by fire. This is less of a problem for oaks since the beginning of active fire suppression throughout their range in the 1950s. Fire is an important part of the maintenance of the structure of Garry oak savannas, and yet remains less common even with our current understanding of its importance (Gilligan and Muir 2011). 3) Garry oak risk being outcompeted by neighboring plants as well as being damaged by herbivory from insects and browsing animals. This is unavoidable in some locations, especially those with encroaching competitive species. It has been shown that thinning of encroaching Doug fir is beneficial to the survival of Garry oak (Devine and Harrington 2013). Due to the lack of predators of Garry oak, the aid of tree shelters, and manual thinning of competitors, some of these issues can be minimized. 4) Garry oak seedlings

have to survive periods of drought during their first season. Drought can be avoided by providing irrigation to the seedlings for their first year (Devine et al. 2007); however, irrigating can be labor intensive and some locations are difficult to visit on a regular basis. Drought is perhaps the most important hurdle that the seedlings have to cross in their first year, particularly on the east side of the Cascade Range where Garry oak often grows on south facing rocky slopes.

The purpose of this investigation is to determine the whether physiological differences, especially in relation to drought-tolerance, exist between several populations of Garry oak from east of, and one population west of, the Cascade Range in Washington State. Differences among physiological parameters for trees, such as maximum photosynthetic rates and stomatal conductance to water vapor were determined for non-stressed as well as water-stressed seedlings. Results of experiments using non-stressed trees will help to determine if some populations have inherently higher photosynthetic rates, and if there are differences in light and water use efficiencies. Results of drought stress experiments will help us determine if there are some populations that can better handle the stress, as well as provide evidence for potential mechanisms that Garry oak employ to deal with limited water availability. This study will provide information to growers about whether collection of acorns to be used in reforestation efforts from specific populations of trees will play a role in survivorship of reforested seedlings. Garry oak savannas are already water stressed at times, and as drought becomes more of a concern due to climate change, the stress could become enhanced. Information from

this study will be useful in making management decisions about this important ecosystem in the Pacific Northwest.

Methods

Acorn Collection and Seedling Growth

Acorns were collected from six locations east of the Cascade Mountain Range in Washington State: Swauk Creek (SW), Kittitas Co. (47°07'25" N, 120°44'04" W); Naches (NC), Yakima Co. (46°44'40" N, 120°47'37" W); Ft. Simcoe (FS), Yakima Co. (46°20'33" N, 120°50'03" W); Goldendale (GD), Klickitat Co. (45°48'24" N, 120°44'44" W); Ahtanum high elevation 1128 meters a.s.l. (AT) Yakima Co. (46°35'13" N, 120°56'98" W) and Ahtanum low elevation 518 meters a.s.l. (AL) Yakima Co. (46°33'56" N, 120°54'14" W). One location west of the Cascade Mountains was also included, Oak Harbor (OH), Island Co. (48°18'05" N, 122°37'47" W). Acorns were collected within 1 meter of a 100 meter transect. A total of 36 seedlings from each population were germinated in 2.83 L tree pots (Stuewe and Sons, Oregon, USA) containing a 6:1:1:2 (v/v) mixture of a peat-based potting soil mix, sand, vermiculite, and perlite. The acorns were germinated in a heated glass greenhouse in mid-October with Sylvania lumalux LU1000, 1000 watt high pressure sodium (HPS) grow lights set on a 16 h day 8 h night cycle. After germination the seedlings were fertilized with Osmocote smart-release® pelletized 14:14:14 NPK fertilizer (The Scotts Co. LLC, Marysville OH), and were inoculated with MycorrhizaROOTS™ ectomycorrhizal mixture (ROOTS Inc., Independence MO). The seedlings were then grown for 90 days to establish roots and develop leaves.

Once established, the seedlings were moved into a climate-controlled room set to 20°C equipped with high pressure sodium (HPS) and metal halide (MH) lights providing photosynthetically active radiation (PAR) of 500 µmol photon m^{-2} s^{-1} set for a 16 h light 8 h dark cycle. The seedlings were watered to saturation weekly or as needed. The seedlings were acclimated to the conditions of the growth room for two weeks prior to the beginning of experimental measurements.

Photosynthetic Response to Light of Non-stressed Populations

Response of photosynthetic CO_2 assimilation (A), stomatal conductance to water vapor (g_s), and photochemical efficiency of photosystem II (PSII) to light (Φ_{PSII}) was measured for three or more randomly chosen seedlings from each population. Light was provided using a 1000 watt MH lamp and a series of neutral density filters to produce light levels of 0, 75, 100, 200, 400, 650, 1150, 1350, and 1500 µmol photon m^{-2} s^{-1} PAR. Carbon assimilation and stomatal conductance rates were measured using a Li-COR 6400 Photosynthesis System (Li-Cor, Lincoln NE) using an ambient (reference) CO_2 concentration of 400 ppm, relative humidity of 30%, and chamber temperature of 23°C. Rapid stomatal responses to variable light were assessed under the same ambient conditions by alternating the light levels from 100 to 1000 and back to 100 µmol photon m^{-2} s^{-1} for a total of three light levels, while recording A and g_s at 1 minute intervals for 25 minutes at every light level. Light adapted photochemical efficiency of PSII (Φ_{PSII}), and maximum photochemical efficiency of PSII (F_v/F_m) were determined according to Genty et al. (1989) using a pulse-modulated fluorometer (FMS1, Hansatech, King's Lynn England).

Morphological and Biochemical Characteristics of Leaves

Seedlings were harvested from each population in order to determine root:shoot ratios (R/S). Shoots and roots were divided into component tissues for each seedling, dried at 70°C to constant mass, and mass measured to determine total aboveground biomass, and total belowground biomass. R/S was determined from dry masses of individual seedlings. Leaf stomatal density (stomates per mm^2) was determined from clear nail-polish impressions of the abaxial epidermal surface of leaves. Garry oak is hypostomatous so only the abaxial surfaces were used. Total chlorophyll concentration (Chl_{total}), specific leaf area (SLA), and relative water content (RWC) were quantified using a 1 cm^2 disk of leaf tissue. Chlorophyll was extracted using 80% (v/v) acetone and quantified spectrophotometrically with a Shimadzu UV-2401 spectrophotometer using extinction coefficients described by Porra et al. (1989). Specific leaf area was calculated as leaf area per unit leaf dry mass ($cm^2\ g^{-1}$).

Induction of Drought Stress and Seedling Response Measurements

Four seedlings from each of the populations AT, AL, NC, SW, and OH were randomly selected for the treatment group and another four for the control. All of the plants were well-watered prior to the beginning of the experiment. Volumetric soil water content (VWC) was measured for the soil in the pots on a daily basis throughout the drought regime using a soil moisture sensor (EC-5 Decagon Devices Inc., Pullman WA). Water was withheld from the treatment group and VWC was either maintained between 5-10% VWC (moderate drought) or withheld entirely for the duration of the experiment (severe drought), up to 42 days. The control group was watered weekly in order to

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maintain a VWC of 20-25%. Measurements including A, g_s, Φ_{PSII}, and F_v/F_m were taken every seven days for the duration of the experiment. Measurements ceased when A for drought stressed plants dropped below 1 µmol CO_2 m^{-2}s^{-1}, which was used as an indication of cellular death within the leaf. All of the plants were exposed to a saturating light intensity of 1000 µmol photon m^{-2} s^{-1} during photosynthetic measurements. Leaf water potential (Ψ_l) was measured using a thermocouple psychrometer (Psypro) attached to a C-52 sample chamber (Wescor Inc., Logan UT) for leaves at the beginning of the experiment (day 0) and four weeks into the drought stress (day 28). Relative water content (RWC) was determined for 1 cm^2 leaf punches as (fresh weight - dry weight) / (turgid weight - dry weight) (100), where turgid weight is the weight of the leaf after equilibration in distilled water for 24 h. Proline was quantified from 100 mg fresh weight of leaf using the ninhydrin assay as described by Ábrahám et al. (2010). Hydraulic conductivity of stems (K_s) was quantified by mass flow rate of a solution through a 2 to 4 cm stem segment (kg s^{-1}) divided by the pressure gradient along the segment (MPa m^{-1}). Stems were cut under water to avoid formation of embolisms in addition to those that may have already been present and were then placed in a tubing apparatus filled with a filtered (0.2 µm) 20 mM KCl solution. Hydraulic conductivity was then measured for the stem using a gravity-induced positive pressure gradient. Maximum hydraulic conductivity for the same stem was then determined by subjecting the stem to repeated flushing with the solution at approximately 175 kPa pressure in order to refill any embolized vessels. Per cent loss of stem hydraulic conductivity (K_s loss) is presented as (maximum conductivity - initial conductivity / maximum conductivity) (100). A detailed description of the procedure used for determining K_s can be found in Sperry et al. (1988).

Field Measurements

Data loggers (Hobo U23-001 Onset computer corporation, Bourne MA) were installed at the acorn collection locations AT, AL, NC, and SW. Relative humidity (RH%) and temperature (°C) were recorded at 12am, 6am, 12pm, and 6pm, every day starting on July 13th 2014 and ending on October 5th 2014 at each of the four sites. These measurements were used to calculate the actual vapor pressure (VPA) and the vapor pressure deficit (VPD) at each location during the warmest and driest days of summer. Measurements of A, g_s, and transpiration (E) under ambient light, vapor pressure, and temperature conditions were made using the Li-COR field-portable gas exchange system for Garry oak seedlings located at each of the sites AT, AL, NC, and SW between the hours of 11am and 2pm on August 15th through 19th 2014. Instantaneous water use efficiency (WUE) was calculated from the measurements as A/E. Seedlings were located within a 100 meter radius of the location of the data logger used to record environmental measurements for that site.

Data Analysis

The parameters A, g_s, Φ_{PSII}, stomatal density, Chl_{total}, RWC, SLA, WUE, VPD, R/S, and proline concentration were analyzed by a two-way analysis of variance (ANOVA). Within group comparisons of control and treatment groups of K_s loss, relative loss of A and g_s, and Ψ_l were analyzed using a Student's t-test. All results were considered significant if $P < 0.05$.

Results

Photosynthetic Response to Light for Non-stressed Seedlings

The photosynthetic response to light from zero to 1150 µmol photon $m^{-2} s^{-1}$ PAR. Photosynthesis from zero to 1500 µmol photons $m^{-2} s^{-1}$ did not significantly differ among Garry oak seedlings from the six eastern Washington populations (AT, NC, AL, SW, FS, GD) and one western Washington population (OH) (Fig 1a, Table 1). However, maximum photosynthetic rates for certain populations of Garry oak were different. Carbon assimilation rates were up to 50% higher for the population with the highest photosynthetic rate (FS) as compared to that with the lowest maximum photosynthetic rate (AT) for light intensities above 1000 µmol photon $m^{-2} s^{-1}$ PAR. Rates of g_s were statistically different across all populations at light levels from 100 to 200 µmol photon $m^{-2} s^{-1}$ and from 1150 to 1500 µmol photon $m^{-2} s^{-1}$ ($P < 0.0001$) (Fig 1b, Table 1). Seedlings from NC maintained the highest g_s at all light intensities and at saturating light, g_s rates were two times higher for NC, as compared to seedlings from the SW population. Instantaneous WUE did not differ across populations from dark to 1500 µmol photon $m^{-2} s^{-1}$ PAR (data not shown). Photochemical efficiency of photosystem II (Φ_{PSII}) was up to 50% lower for AT seedlings as compared to other populations (Fig. 1c) but differences among populations were only significant at 1150 to 1500 µmol photon $m^{-2} s^{-1}$ at which point Φ_{PSII} for FS and SW was more than 35% higher than AT (Table 1).

Table 1. Photosynthetic response to light. Steady state measureents measured at 1150 and 200 μmol photon m^{-2} s^{-1}. Average A (μmol CO_2 m^{-2} s^{-1}) $\pm$ SE and average g_s (mmol H_2O m^{-2} s^{-1}) $\pm$ SE (n=3). Average Φ_{PSII} is in relative units $\pm$ SE (n=3). Differences denoted by (a) and (b) are based on an ANOVA ($P < 0.05$) followed by a Tukey's HSD test.

| Population	$A_{(200)}$	g_s $_{(200)}$	$\Phi_{PSII(200)}$	$A_{(1150)}$	g_s $_{(1150)}$	$\Phi_{PSII(1150)}$
AT	3.65 ± 0.38	0.100 ± 0.013 a	0.655 ± 0.015	10.25 ± 0.94	0.165 ± 0.023	0.350 ± 0.040 a
NC	5.40 ± 0.24	0.20 ± 0.013 b	0.699 ± 0.018	13.03 ± 1.59	0.301 ± 0.063	0.509 ± 0.030 ab
AL	4.49 ± 0.43	0.120 ± 0.011 ab	0.680 ± 0.015	12.73 ± 1.77	0.253 ± 0.031	0.438 ± 0.028 ab
SW	5.76 ± 0.44	0.090 ± 0.019 a	0.724 ± 0.013	12.33 ± 1.57	0.136 ± 0.023	0.541 ± 0.061 b
OH	4.14 ± 0.98	0.071 ± 0.012 a	0.726 ± 0.012	12.07 ± 0.74	0.182 ± 0.044	0.537 ± 0.024 ab
FS	5.21 ± 0.42	0.187 ± 0.006 ab	0.715 ± 0.033	14.16 ± 1.62	0.277 ± 0.018	0.548 ± 0.047 b
GD	4.31 ± 0.19	0.135 ± 0.013 ab	0.702 ± 0.010	10.58 ± 0.70	0.243 ± 0.010	0.457 ± 0.030 ab
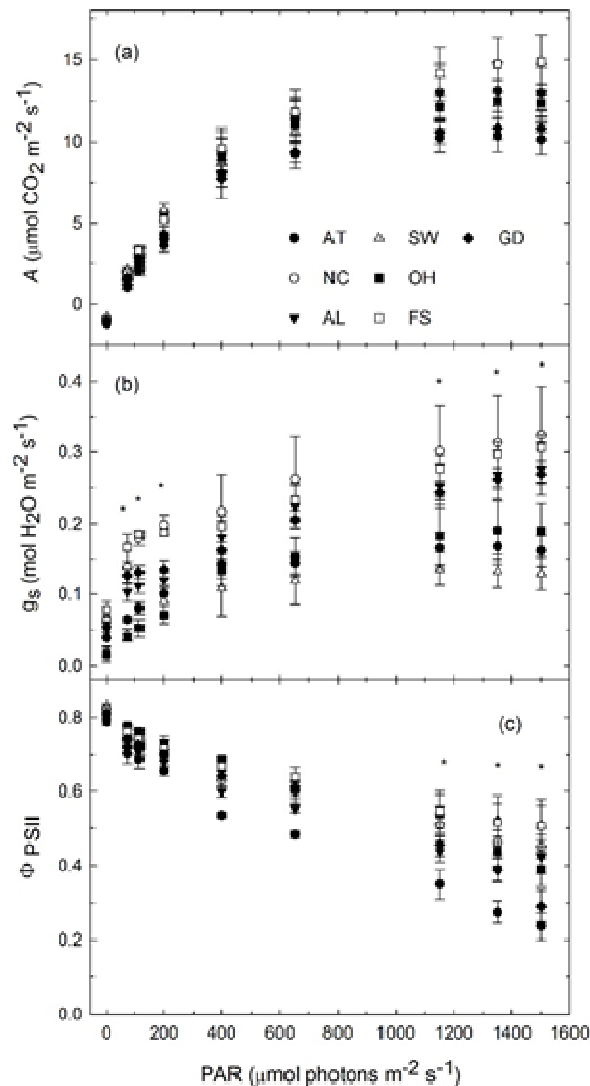
Figure 1. Response of non-stressed seedlings to light. Symbols represent average (n=3, ±SE). (a) Photosynthetic response to light of A for seven populations of Garry oak. No significant differences were observed between populations. (b) Photosynthetic response of g_s for seven populations of Garry oak. Differences were statistically significant at light intensities at and below 200 and above 1150 μmol photon m^{-2} s^{-1}. (c) Response of photochemical efficiency of PSII for seven populations of Garry oak. Differences were statistically significant at light intensities at and above 1350 μmol photon m^{-2} s^{-1}. * indicates difference based on an ANOVA ($P < 0.05$) followed by a Tukey's HSD test.

Stomata from all populations were highly responsive to rapid changes in light intensity. Upon exposure to a light intensity of 1000 µmol photon m^{-2} s^{-1}, stomatal conductance and assimilation rates rapidly increased to nearly steady-state rates within two min (Fig. 2). Upon subsequent exposure to low light (100 µmol photon m^{-2} s^{-1}), stomatal conductance and assimilation rates for the same leaf decreased by 3.7 and 4.5 times, respectively within four min. Repeated exposure of seedlings to light or dark cycles, or exposure of leaves to varied air VPD produced a similar rapid stomatal response (data not shown).
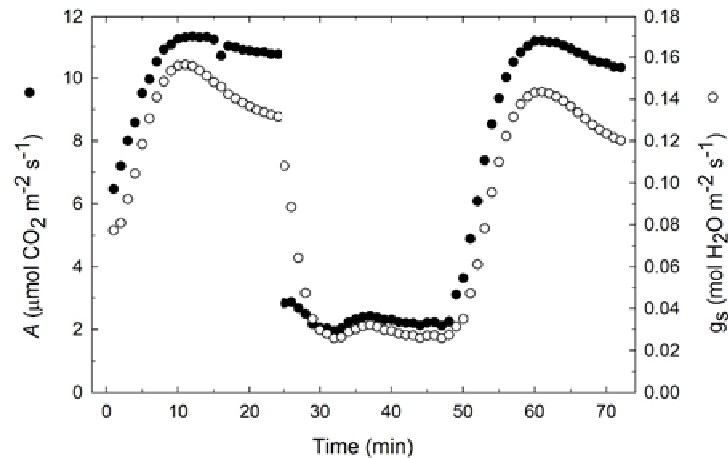
Figure 2. Rapid stomatal response to light. Response of A (●) and g_s (□) to alternating high and low light intensity for Garry oak seedlings from AT. Light levels were held at 1000 μmol photon m^{-2} s^{-1} for 25 minutes, switched to 100 μmol photon m^{-2} s^{-1} for 25 minutes, and back to 1000 μmol photon m^{-2} s^{-1} for 25 minutes. Measurements of A and g_s were made every minute.

Anatomical and Biochemical Properties for Leaves from Non-stressed Seedlings

Stomatal density for leaves was highly variable within seedlings from all populations of Garry oak sampled and, frequently, differed by 10% from population to population. OH had a stomatal density that was 30% higher compared to the other

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populations that were measured (Table 2). Specific Leaf Area (SLA) was also variable

across populations and was significantly larger for SW compared to the populations AT,

NC, and AL ($P < 0.01$). Leaves from SW and OH seedlings had higher total chlorophyll

concentration when calculated on an area basis but not when calculated on a leaf mass

basis (Table 1) due to an increase in leaf thickness for leaves from these populations.

Seedlings grown from acorns collected at OH and SW were generally but not

significantly larger than those from other populations in higher shoot and root dry weight

(data not shown), and there were no significant differences in R:S ratio for seedlings

across populations (Table 2).

Table 2. Morphological and biochemical characteristics. Average stomatal density (stomates mm^{-2}) ± SE (n=3). Average specific leaf area ($mm^2\ mg^{-1}$) ± SE (n=6). Average chlorophyll concentration ± SE were determined from 1 cm^2 leaf disks (n=3), and presented on an area basis ($\mu mol\ cm^{-2}$), and on a mass basis ($\mu mol\ mg^{-1}$). Average root:shoot ratios ± SE were calculated from total dry weight of component tissues (n=3). Differences denoted by (a) and (b) are based on an ANOVA ($P < 0.05$) followed by a Tukey's HSD test.

Population	Stomatal Density	SLA	$Chl_{total}\ Area^{-1}$	$Chl_{total}Mass^{-1}$	R/S
AT	350 ± 15.0 a	7.83 ± 0.39 a	15.5 ± 3.0 a	1.44 ± 0.58	5.08 ± 1.49
NC	360 ± 13.0 a	7.66 ± 0.61 a	17.6 ± 3.9 a	1.29 ± 0.67	4.74 ± 0.65
AL	329 ± 10.4 a	7.97 ± 0.20 a	21.0 ± 2.0 ab	1.78 ± 0.19	3.23 ± 0.22
SW	340 ± 19.4 a	9.76 ± 0.38 b	31.6 ± 2.3 b	2.96 ± 0.26	3.09 ± 0.09
OH	437 ± 10.6 b	8.21 ± 0.27 ab	27.5 ± 3.3 b	2.35 ± 0.37	4.68 ± 0.72

The soil water capacity for the seedlings in the control group remained at an average of 21.4% ± 0.53 VWC throughout the 42-day experiment (Fig. 3). Soil water content for droughted pots fell to 75% of control soil water content by day seven in each experiment and to 50% of control soil water content by day 14. The soil water content for the moderate drought stress remained at about 50% of control values until day 42 where it dropped to 25% of the control. By day 28, soil for the severe drought stress plants was at a water content that was less than 25% of control soil water content and by day 42, when assimilation rates for most plants were close to zero, water content for the soil in the severe drought treatment was less than 10% of the control soil water content (Fig. 3).
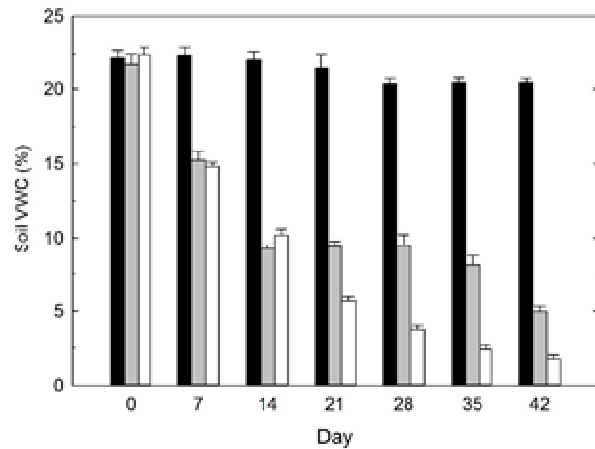
Figure 3. Soil water content for drought stress treatments. Water content for the watered control plants (solid bars) was maintained near 20% (v/v). Water content for moderate drought stressed plants (shaded bars) was maintained near 10% (v/v) after 14 days. Water was completely withheld from the drought stressed plants for the severe drought stress experiment (empty bars). Bars represent soil VWC means ±SE (n=4).

Light-saturated rates of A and g_s decreased by less than 10% of control rates for Garry oak seedlings exposed to the 42 day moderate drought stress experiment (data not shown). Instantaneous WUE also remained unchanged for seedlings exposed to 42 days of moderate drought stress. For seedlings exposed to severe drought stress, light-saturated A and g_s rates for SW seedlings dropped to 65% of control at 2 weeks and 40%

of control rates at 3 weeks (Fig. 4a, Fig. 4b). At the fourth week of exposure to severe

drought, rates of A and g_s were significantly lower than control rates for Garry oak

seedlings from all populations that were exposed to drought stress. Rates for SW

continued to be the lowest as compared to other Garry oak populations, but there was no

significant difference in the response of different populations of Garry oak to drought

stress for weeks four to six of the drought stress treatment. Although rates of A and g_s

varied for seedlings during this time, instantaneous WUE was not variable across

populations or between control and droughted leaves for the course of the drought

treatment (Fig. 4c). By week five, all of the SW seedlings exposed to severe drought

exposure had died. Rates of A and g_s were relatively higher for OH and NC as compared

to rates for AT, and instantaneous WUE was markedly higher for OH seedlings at this

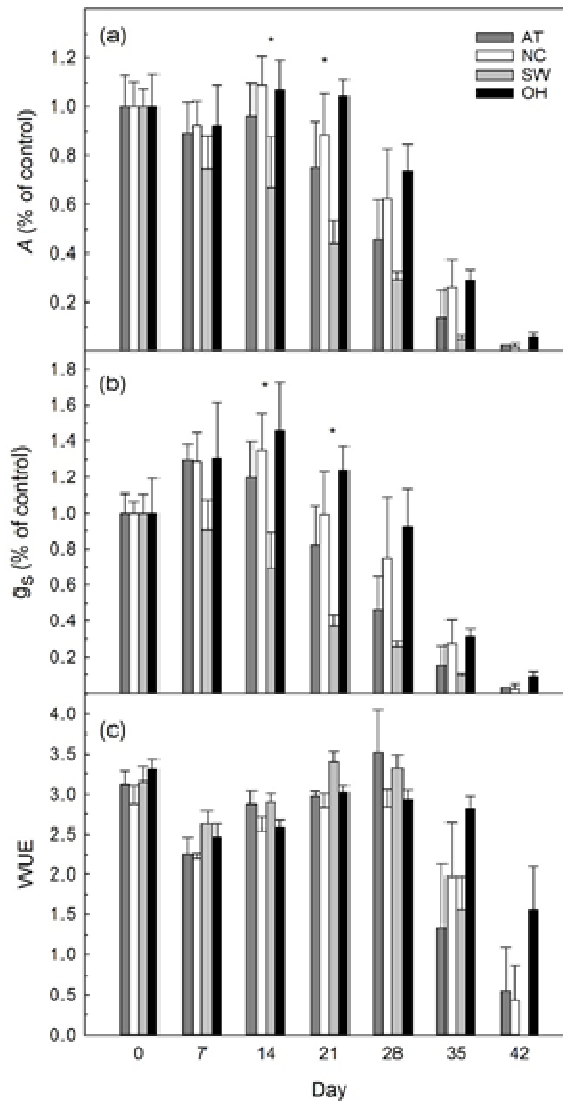time, but these differences were not significant.

Figure 4. Response of Garry oak seedlings to drought. (a) Steady state A measured at 1000 µmol photon m^{-2} s^{-1} for the stressed plants during the 42 days of severe drought. (b) Steady state g_s measured at 1000 µmol photon m^{-2} s^{-1} for the stressed plants. * indicate differences based on an ANOVA ($P < 0.05$) followed by a Tukey's HSD test. (c) Instantaneous water use efficiency calculated from A/E measurements under the same conditions.

Carbon assimilation decreased as VWC decreased for all populations of Garry

oak seedlings exposed to severe drought stress. From two to five per cent VWC, which

was the point at which the soil dried enough that seedlings from all populations began to

die. Seedlings from the OH population, a population that grows on the west side of the

Cascade mountain range, had rates of assimilation that were up to two times higher than

rates of assimilation for the three populations from the east side of the cascade mountain

range (Fig. 5). Assimilation rates for SW were consistently lower than for other

populations over the same range of VWC (Fig. 5). No differences in F_v/F_m or Φ_{PSII}

between control and droughted seedlings or across populations of Garry oak in response

to drought were observed (data not shown). Likewise, exposure to drought did not

change leaf anatomical or growth parameters for Garry oak seedlings such as SLA or R:S
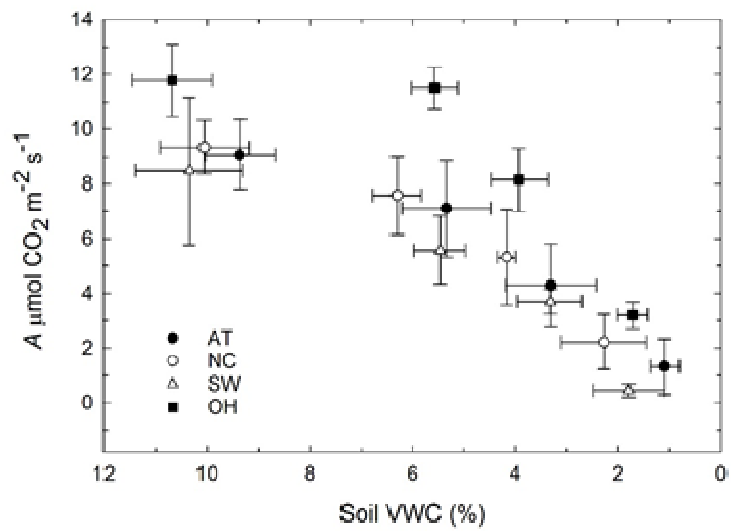
(data not shown).

Figure 5. Response of seedlings to soil water content. Symbols represent average values ±SE (n=4). Oak harbor maintained the highest assimilation rates at low available water.

Few changes in growth or biochemical characteristics in Garry oak seedlings

occurred in response to drought. Leaf proline content increased slightly in response to

drought for seedlings from all populations of Garry oak, with the exception of the OH

population (data not shown). The increase in proline concentration for the AT

population, however, was more than 6 times for leaves of droughted plants as compared

to control plants (0.83 ± 0.095 mg g^{-1} FW for control, 5.08 ± 0.90 for droughted). This

increase in proline content for AT leaves upon exposure to drought did not change the Ψ_1

or RWC for AT leaves as compared to leaves from three other populations of Garry oak

upon exposure to severe drought (Fig. 6). Drought exposure lowered the average leaf Ψ_1

by at least 0.8 MPa with an average change of 0.98 ± 0.15 MPa as compared to leaf Ψ_1

for control seedlings for all populations. However, this decrease was only significant for

AT and SW populations (Fig. 6a). Likewise RWC was generally lower by at least 10%

for leaves of droughted, as compared to control plants but this difference was only

significant for leaves from AT, and OH populations (Fig. 6b).
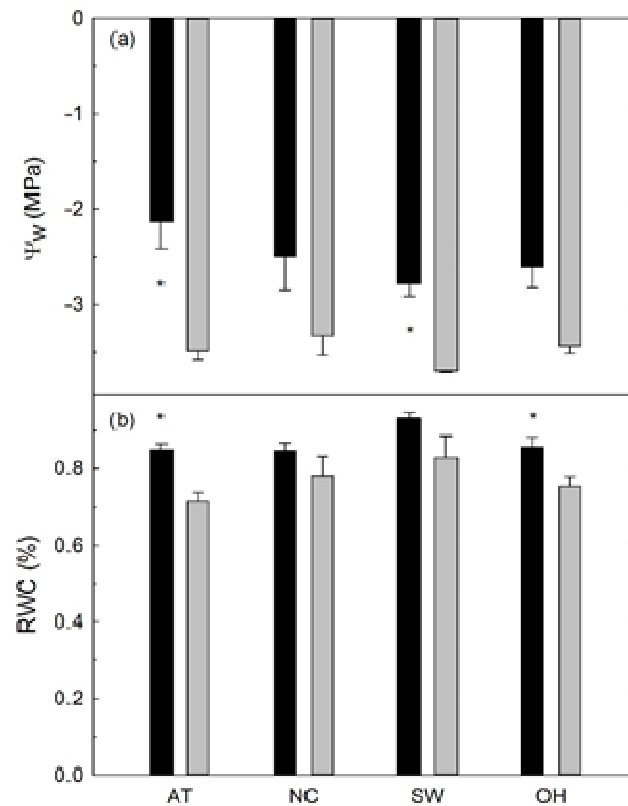
Figure 6. Water potential and relative water content response. (a) Bars represent average leaf water potential (±SE) for Garry oak seedlings (n=4). (b) Bars represent average leaf relative water content (±SE) for Garry oak seedlings measured from 1 cm^2 leaf punch (n=4). Solid bars represent control plants. Shaded bars represent stressed plants. * indicates difference between control and treatment groups based on a Student's t-test ($P < 0.05$).

Relative loss of stem hydraulic conductivity for seedlings from NC and AT populations of Garry oak was up to four times higher after exposure to 42 days of severe drought as compared to loss of hydraulic conductivity for seedlings not exposed to drought (Fig. 7). Other populations also exhibited a decrease in hydraulic conductivity in response to drought but these differences were not statistically significant. Figure 8 shows a comparison of the loss of hydraulic conductivity as a function of Ψ_l for four populations of Garry oak exposed to a severe drought treatment. While all populations incurred a loss of hydraulic conductivity in response to drought, plants from the SW population were able to withstand much lower Ψ_l with relatively less loss in hydraulic conductivity (47.5% ± 18.9%) as compared to seedlings from AT, OH and NC populations. In contrast, NC had a relatively high loss of hydraulic conductivity (75% ± 11.9%) at a Ψ_l and RWC (%) that was not significantly different from that for control plants.
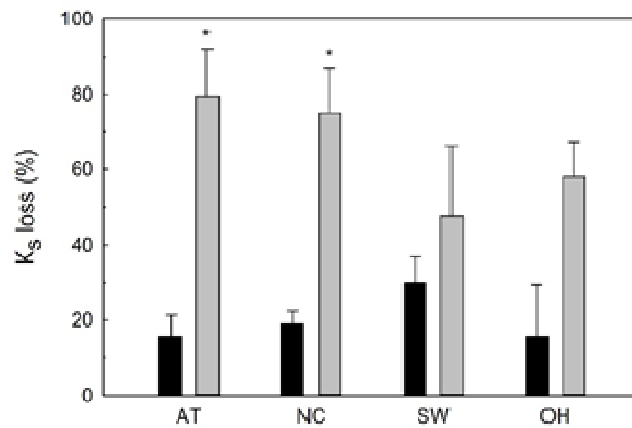
Figure 7. Response of stem hydraulic conductivity to drought. Bars represent average relative loss of hydraulic conductivity (±SE) for the watered (solid bars), and drought stressed (shaded bars) plants (n=3). *indicates difference between control and treatment groups based on a Student's t-test ($P < 0.05$).
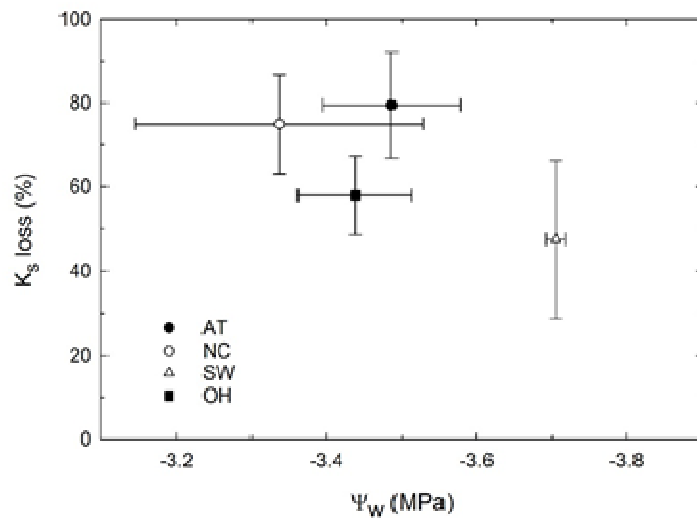
Figure 8. Response of Hydraulic Conductivity to
Water Potential. Symbols represent average
relative loss of stem hydraulic conductivity ($\pm$SE)
at decreasing average leaf water potential ($\pm$SE)
for the stressed plants, after four weeks of drought
(n=4).

Field Measurements

Field measurements of *A* and g_s indicated that NC had assimilation rates that were more than 50% lower than for AL, NC or SW measured in the field and that field-measured stomatal conductance rates were more than 50% lower for NC and AT population as compared to rates measured in the laboratory. SW had *A* and g_s rates that were more than 75% higher than NC ($P < 0.05$, n $\geq$ 7). The seedlings at AT had instantaneous WUE values measured in the field that were similar to that measured in the laboratory (3.59 ± 0.4, n=19) while field-measured instantaneous WUE for other populations were lower than those measured in the laboratory (Table 3). Average temperature, vapor pressure of the air (VPA) and vapor pressure deficit (VPD) measurements for four eastside locations as recorded from July 13th to October 5th 2014 are shown in Table 3. Mid-day VPD was calculated for all four locations from the data logger measurements. NC had the highest average temperature and VPD. The VPD at NC was more than 25% higher than for AT or SW. NC also had the highest mid-day VPD which was more than 45% higher than the other three populations ($P < 0.001$, n=84).

Table 3. Field photosynthetic and microclimate measurements. Average instantaneous water use efficiency WUE (± SE) was calculated from average A (μmol CO_2 m^{-2} s^{-1}) ± SE and E (mmol H_2O m^{-2} s^{-1}) ± SE measurements taken from Garry oak seedling growing at the acorn collection sites along with average g_s (mmol H_2O m^{-2} s^{-1}) ± SE (n≥7) Average temperature (°C) ± SE, average actual vapor pressure VPA (Pa) ± SE, and average vapor pressure deficit VPD (Pa) ± SE were calculated from four measurements taken per day by Onset Hobo data loggers between July and October 2014. Differences denoted by (a) and (b) are based on an ANOVA $P < 0.001$ followed by a Tukey's HSD test.

Population	A	g_s	WUE	T°C	VPA	VPD	VPD$_{mid-day}$
AT	5.13 ± 0.96 ab	0.051 ± 0.012 a	3.59 ± 0.4 a	17.6 ± 0.4 a	924.0 ± 15.2 a	1244.9 ± 52.1 a	2590.3 ± 77.7 a
AL	5.92 ± 0.92 ab	0.113 ± 0.019 ab	1.07 ± 0.06 c	17.3 ± 0.5 a	1110.8 ± 19.2 a	1115.2 ± 64.4 a	2943.0 ± 81.9 b
NC	1.60 ± 0.65 a	0.028 ± 0.009 a	1.41 ± 0.44 bc	22.7 ± 0.5 b	1026.9 ± 18.0 bc	2094.7 ± 83.8 b	4358.1 ± 104.3 c
SW	6.84 ± 0.74 b	0.128 ± 0.020 b	2.20 ± 0.13 b	19.2 ± 0.4 c	1007.1 ± 14.9 c	1411.3 ± 57.1 a	2911.0 ± 78.2 b

Discussion

Comparison of Non-Droughted Populations of Garry Oak

There was a high amount of variation between rates of A, g_s and instantaneous WUE for individual seedlings within populations of Garry oak measured in the laboratory and in the field. This variation between seedlings made it difficult to discern significant differences in assimilation or stomatal conductance rates at saturation levels of light across different populations. However, comparisons between populations indicate that some eastern populations of Garry oak potentially have higher maximum light-saturated rates of A and g_s than others, furthermore photosynthetic rates of the eastern populations were similar to rates for the western population of Garry oak that was investigated. Similarly, A_{max} and g_{smax} differed for European oaks (*Quercus petraea*) from populations at higher versus lower elevations, but intrapopulation variability was much higher than interpopulation variability (Bresson et al. 2011). Results of the present study indicate that considerable intrapopulation and interpopulation variability in photosynthetic parameters also exist for Garry oak.

Significant differences in stomatal conductance rates observed at relatively low light (200 µmol photon m^{-2} s^{-1}) may affect WUE, and ultimately survival for Garry oak seedlings in shaded understory conditions that commonly occur in Garry oak woodlands and savannas (Brose 2011). Decreased WUE that results from higher rates of g_s for populations such as NC and FS under shaded conditions may be a disadvantage for seedlings establishment in habitats that are water and light-limited. Of all of the populations examined in this study, AT and SW are the most conservative in terms of

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light and water use having lower A_{max} and g_{smax} rates than the other populations measured. AT and SW are also capable of a high degree of down-regulation of the photochemical efficiency of PSII at high light, indicating that these populations are capable of avoiding photoinhibitory damage to PSII that might occur in conditions of high light and low water availability. On the contrary, NC and FS have some of the highest light-saturated A and g_s rates and exhibit the least amount of down-regulation of the efficiency of PSII at high light, giving them potentially higher growth rates in the field, possibly at the cost of being more susceptible to light-induced damage under drought conditions

Bur oak (*Quercus macrocarpa*) has a strong rapid stomatal response to a change in light, much greater than other trees in the same ecosystem (Knapp 1992). Despite the observation that plants with a lower A_{max} usually have a lower degree of stomatal response as compared to plants with higher A_{max}, bur oak leaves have responsive stomata regardless of A_{max} for the leaf (Hamerlynck and Knapp 1994). Bur oak is related to Garry oak as they both belong to the subgenera *Lepidobalanus* (White oaks) sensu Camus (Bonner and Vozzo 1987) and all populations of Garry oak used in this study exhibited rapid response of A and g_s to light (Fig 2). Such rapid responses of stomates to light, effectively moderate the decreases in Ψ_l during high light events for bur oak, increasing drought tolerance for this species. Rapid stomatal response to light as measured for seedlings from all of the populations investigated in this study are a useful water-saving adaptation for continual drought conditions Garry oak seedlings are exposed to in their normal geographic range.

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Differences in leaf chlorophyll concentration among populations indicates the ability for Garry oak to acclimate to different light levels in the oak stands. Total leaf chlorophyll content in *Quercus robur* varies in response to insect attack, drought, and pollution and can be used as an indicator of declining forest condition (Rossini et al. 2006). However, since the oak seedlings used for this study were all grown with the same light regime, the differences in leaf chlorophyll concentration indicate potential genetic differences in biochemical properties of leaves from different populations rather than a response to contrasting light environments. In the present study, chlorophyll concentration for Garry oak differed across populations when presented on an area basis but not when presented on a weight basis, indicating that differences were possibly due to leaf morphology. Populations with higher chlorophyll per area also had higher SLA. This trend is especially clear and statistically significant when comparing SW to AT, and NC. Leaf anatomical properties, including stomatal density and leaf thickness tend to be more plastic for more drought tolerant species of oaks, such as *Q. velutina*, and less plastic for less drought-tolerant oaks such *Q. rubra*. Furthermore species of oaks that are more drought tolerant tend to have a higher stomatal density but lower stomatal area (Ashton and Berlyn 1994). Here we found a high amount of intrapopulation variability in stomatal density and a significant difference between OH and all other populations, with OH having on average 20% higher stomatal density.

Physiological Response of Garry Oak to Drought

Measurable differences in g_s and *A* among the response of populations of Garry oak exposed to a severe drought appeared after 14 days of exposure to drought (Fig.4).

This time frame coincided with a soil water content of about 10% which was half the soil VWC for control seedlings. SW exhibited the fastest decrease in A which was due largely to a stomatal limitation of photosynthesis as no decrease in maximum photochemical efficiency of droughted plants was observed. The seedlings from SW were the first to succumb to drought. All of the SW seedlings perished after 31 days. Previous drought experiments for red oak (*Quercus rubra*) did not show a decline in stomatal conductance after 14 days of withholding water (Osonubi and Davies 1978) and although SW was the only population of Garry oak that responded to drought through reduction of A and g_s after 14 days, three other populations began a decline in these photosynthetic parameters after 21 days and all populations measured had markedly lower photosynthetic rates after 28 days. Regardless of the population, the threshold for survivability of water stress was between 30 to 40 days. AT, NC, and OH maintained positive A rates for up to 42 days and a soil water content less than 2% (v/v). This suggests that as a general rule, Garry oak seedlings can withstand 30 days of withholding water when in moderate sub-saturating light intensity, and is consistent with previous drought experiments in which container grown pubescent oaks (*Quercus pubescens*) survived 37 days of withheld water (Gallé et al. 2007). The populations from the western side of Washington (OH) maintained the highest rates of A and instantaneous WUE of all populations throughout the drought exposure (Fig. 4), and also maintained markedly higher A in conditions of low volumetric water than the other three populations (Fig 5). The observation that a population from the more mesic side of Washington state (OH) maintained higher photosynthetic rates throughout the drought exposure, while the population from the most northern of the populations of Garry oak that occur on the

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eastern side of Washington (SW) was the least tolerant of drought exposure, might be explained by factors such as differences in the types of soil and fresh water availability at the two locations. SW is the northernmost population of Garry oak that occurs on the east side of the Cascade Mountain range. We expected that plants from more arid environments would better survive drought stress. Whidbey Island is located in the Puget Sound and while it is not an arid or semi-arid environment, still receives summer drought along with sandy soil conditions that may in fact provide a harsher moisture environment than exists for some of the eastern populations. This may especially be the case in populations such as SW that are located relatively close to a freshwater creek. In addition, Whidbey Island oaks are genetically more closely related to Garry oak stands in southern California than they are to nearby Pacific Northwest populations (Ritland et al. 2005).

Reversible down regulation of PSII was observed in *Quercus pubescens* growing in the field during the daytime of drought periods (Haldimann et al. 2008) and in response to heat shock up to 52°C (Dascaliuc et al. 2007). Changes in photochemical efficiency for Garry oak without a loss of maximum photochemical efficiency (F_v/F_m) indicates that photosynthetic reaction centers of the seedlings were not damaged during the drought but rather seedlings were able to respond to high light and effectively protect themselves from light-induced damage during a drought. Seedlings from all populations responded to drought through decreased stomatal conductance rates that facilitated instantaneous WUE values that were relatively unchanged throughout more than a month of severe drought.

All Garry oak seedlings showed a slight decrease in leaf water potential in response to drought (Fig. 6). We expected the change of leaf water potential to correlate with proline accumulation in the leaves and to offset loss of RWC and potentially increase photosynthetic performance for seedlings. The change in leaf water potential for AT was indeed paired with an increase in proline concentration, but this was not the case for SW which had a similar final water potential and decreased RWC that was not associated with proline accumulation. Other compounds and solutes may have been responsible for changes in water potential observed in seedlings from other populations. In prolonged drought studies it has been shown that shoot starch concentration decreased while soluble sugar increased after severe drought (Villar-Salvador et al. 2004). Another potentially important intracellular factor in protection against drought is the relative abundance of protective proteins such as dehydrins. Dehydrin expression has been shown to change in response to drought in English oak (*Quercus robur*) (Šunderlíková et al. 2009). In sessile oak (*Q. petraea*) there is a high degree of biochemical variation with elevation gradients including differences in occurance of dehydrins (Vornam et al. 2011). A greater understanding of the balance of solutes and proteins in the droughted seedlings of Garry oak is needed in order to make any conclusions about the mechanisms behind the water potential changes.

The significant loss of hydraulic conductivity for Garry oak seedlings from the AT and NC populations, and the lack of a similar loss in the SW and OH population was contrary to our expectation based on lower survivorship of SW in response to drought. SW exhibited a relatively low loss of hydraulic conductivity at relatively large xylem

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tensions based on Ψ_1 measurements, in comparison to seedlings from other eastside populations (Fig.8). Yet OH, a population from the more mesic west side of the Cascades were also relatively unaffected by drought in terms of loss of hydraulic conductivity. A possible explanation for this anomaly may be interpopulation variation in the stem diameter of the seedlings. Seedlings with smaller xylem conduit diameter can better withstand drought whereas recovery from drought-induced cavitation occurs at a lower positive root pressure for vessels with wide diameters than for smaller vessels (Logullo and Salleo 1993). Neither Stem nor vessel diameter were measured quantitatively in this study, however, visible differences were observed between the populations, with OH and SW having relatively larger, and NC and AT relatively small diameters. Adult Garry oak trees, with a higher ratio of leaf to sapwood area as compared to seedlings, leads to even further hydraulic limitations to water flux than for seedlings (Phillips et al. 2003) so drought-induced loss of hydraulic conductivity for adult trees may be of greater importance than for the seedlings of these trees growing in the field.

Field Measurements

Despite the fact that no significant differences were observed for light-saturated photosynthetic rates for seedlings across populations measured under laboratory conditions, field measurements showed significantly lower (NC) and higher (SW) rates of A_{max} and g_{smax} as compared to rates in the field measured for AL and AT populations. AT had a field instantaneous WUE that was comparable to that seen in laboratory conditions, but was also more than 25% higher than any other location as measured in the field. This

was due to a relatively high A along with relatively low g_s. This is an indication of plants that have developed and grown in prolonged drought and that have stomata that rapidly respond to differences in sunlight and water availability. Drought adapted oaks generally have more sensitive stomatal response and root to shoot ratio shifts that maximize the efficiency of water use (Thomas and Gausling 2000, Xu and Baldocchi 2003). Some oaks have been shown to exhibit patchy stomata during times of drought. These plants operate independent patches of stomates across the leaf in order to maximize the use of CO_2 while minimizing water loss (Epron and Dreyer 1993). Significant differences in instantaneous WUE were not observed in the artificially droughted container grown plants measured under steady-state conditions. The difference in photosynthetic response of populations growing in the field versus in a laboratory setting were most likely a result of the extreme VPD that field seedlings were exposed to. The controlled VPD of the container grown seedlings was constantly held near 2000 Pa, while the VPD at the collection sites was in some cases more than double that value. Soil water content and leaf water potential were not measured for the field grown seedlings but results indicate that non-steady state responses to light and water availably are dramatically important for Garry oak photosynthesis and growth in the field. It is also well understood that below-ground responses of *Quercus* species such as changes in root growth patterns are equally important to above ground responses such as decreased stomatal conductance rates when tress are exposed to drought (Manes et al. 2006). Root morphology is an important determiner for survivorship of Garry oak seedlings used for reforestation efforts (Gould and Harrington 2007). Further investigations on non-steady-state characteristics of photosynthesis and growth responses of seedlings growing in the field to changes in

environmental parameters are needed in order to reveal the strategy for drought-tolerance of these seedlings.

Conclusions

Concerning acorn collection for restoration efforts east of the cascades, several factors should be considered. Of the east side microclimates measured, NC was the most severe in regard to high VPD. This was the harshest environment measured in consideration of seedlings exposure to high temperatures and low water availability. While not located at the harshest microclimate, seedlings from AT had the highest WUE measurements in the field, which was comparable to what they maintained in the laboratory setting potentially making this an ideal site for seed collection. AT also showed the potential for a strong leaf water potential response to drought, either from proline accumulation or through another mechanism. AT showed a decreased Φ_{PSII} with increasing light but no reduction of F_v/F_m which suggests that they are able to protect themselves from light-induced damage even under conditions of limited water availability. Seedlings from SW were the most variable in assimilation measurements and the most susceptible to drought in the water stress experiment. This suggests that SW might not be an ideal collection site for acorns. The susceptibility to embolism induced loss of hydraulic conductivity makes AT and NC less ideal for dry site plantings, whereas if hydraulic conductivity maintenance in response to drought is the primary consideration then OH would be better suited. However, loss of hydraulic conductivity in response to freezing damage has also been shown to be an important determiner for survivorship of holm oak (*Quercus ilex*) (Logullo and Salleo 1993) and several white oak

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species from the southeastern US (Cavender-Bares and Holbrook 2001) and susceptibility to freezing stress may preclude the use of seeds from Western populations of Garry oak for reforestation efforts on the Eastern side of the state (Huebert 2009). Our results suggest that there are physiological differences between the populations, but also a great deal of intrapopulation variation suggesting that individuals from any of the populations may potentially be ideal for reforestation efforts. Continued research will further benefit reforestation efforts by providing insight into the physiological and biochemical variations in and among the populations of Garry oak east and west of the Cascades, as well as the thresholds for drought stress tolerance for Garry oak seedlings.

Conflict of Interests

None declared

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