AN ABSTRACT OF THE DISSERTATION OF

Mingyang Liu for the degree of Doctor of Philosophy in Crop Science presented on October 27, 2016.

Title: Response of Roughstalk Bluegrass (*Poa trivialis* L.) to Waterlogging Stress

Abstract approved:

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Roughstalk bluegrass (*Poa trivialis* L.) (RB) is a weed species that increased very quickly in grass seed production fields of the Willamette Valley. The invasion of RB in grass seed crops often occurs in waterlogged soils. In waterlogged areas, RB exhibited better competitiveness and had greater population densities than other grass species. Oxygen deficiency is the major environmental stress caused by waterlogging. Studies were conducted to investigate the physiological characteristics that potentially contribute to the better waterlogging tolerance of RB. Oxygen deficiency resulted in a germination delay in both RB and tall fescue (*Festuca arundinacea* Schreb.) (TF) species, but the delay for TF was greater than for RB. Higher temperatures (20 and 30 C) increased the influence of oxygen deficiency on TF, but did not influence the effect of oxygen deficiency on RB. In a greenhouse study, 28 days of waterlogging during the early establishment stage reduced the aboveground biomass of RB and TF by 58 and 46%, respectively, but did not influence seedling numbers. Alcohol dehydrogenase (ADH) activity in both RB and TF seedlings increased under anoxic treatment. After 4 weeks of

waterlogging treatment, the leaf number and plant height were reduced more in TF than in RB. The leaf number was reduced by 32 and 30% in TF and RB, respectively. The plant height was reduced by 42 and 35% in TF and RB, respectively. The root dry biomass was reduced by 43 and 13% in TF and RB, respectively. The root length increased 6% in waterlogged TF, and decreased 42% in waterlogged RB. Compared to TF, RB root system was shallower after four week long waterlogging treatment. At 1, 2, 3, and 4 weeks after treatment, ADH activity in root of mature plants increased 93, 45, 39, and 57% in waterlogged TF, and increased 56, 27, 22, and 23% in waterlogged RB. At 1, 2, 3, and 4 weeks after treatment, lactate dehydrogenase (LDH) activity in roots of mature plants increased 15, 18, 13, and 13% in waterlogged TF, and 2, 19, 5, and 11% in waterlogged RB. The turf quality, aboveground biomass, photosynthetic capacity, and water soluble carbohydrate concentrations were reduced by the waterlogging treatment, but the reductions were not different between RB and TF. RB has a larger root aerenchyma areas than TF. After a four week waterlogging treatment, the aerenchyma by area portions were 23 and 34% in drained and waterlogged TF roots, and 29 and 38% in drained and waterlogged RB roots. Root porosities increased in both waterlogged TF and RB, but the increases were not different between TF and RB. After a four week waterlogging treatment, the root porosities were 25 and 31% in drained and waterlogged TF plants, and 23 and 30% in drained and waterlogged RB plants. Physiological characteristics including quicker seed germination, lower oxygen required during establishment, a thicker and shallower root system, lower fermentation under low oxygen stress, and larger aerenchyma areas may contribute to better waterlogging tolerance in RB.

Response of Roughstalk Bluegrass (*Poa trivialis* L.) to Waterlogging Stress

by
Mingyang Liu

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented October 27, 2016
Commencement June 2017

Doctor of Philosophy dissertation of Mingyang Liu presented on October 27, 2016

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Mingyang Liu, Author

ACKNOWLEDGEMENTS

First and foremost, I want to thank my co-major professors, Dr. Carol Mallory-Smith and Dr. Andrew Hulting. They have taught me, both consciously and unconsciously, how good experimental research is done. I appreciate all their contributions of time, ideas, and funding to make my Ph.D. experience productive and stimulating.

I would also like to thank my committee members, Dr. Alec Kowalewski, Dr. Ricardo Mata-Gonzalez, and Dr. Jeremiah Dung for serving as my committee members even when they are busy. I also want to thank them for letting my study be an enjoyable experience, and for their brilliant comments and suggestions.

The studies in this dissertation would not have been possible without the support from Dan Curtis, Kyle Roerig, Suphannika Intanon, and Nami Wada. I have appreciated their help in providing experimental materials and suggestions.

The members of the weed group have contributed immensely to my personal and professional time at Oregon State University. The group has been a source of friendships as well as good advice and collaboration. I would like to acknowledge all the past and present fellow students and group members that I have had the pleasure to work with or alongside.

I would like to extend my thanks to Department of Crop and Soil Science and all my friends at Oregon State University. My time at Corvallis was made enjoyable in large part due to the many friends and groups that became a part of my life.

Lastly, I would like to thank my family. Words cannot express how grateful I am to my parents for all of their encouragement and support that they have made on my behalf. I would also like to thank to my wife Yulin Sun, my daughter Lisa Liu, and my son George Liu, who always cheer me up.

CONTRIBUTION OF AUTHORS

Dr. Carol A. Mallory-Smith and Dr. Andrew G. Hulting proposed and advised all the aspects of this research. Dr. Carol A. Mallory-Smith and Dr. Andrew G. Hulting were also involved in the improvement of the manuscripts.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

Figure Page

LIST OF FIGURES (Continued)

Figure Page

LIST OF FIGURES (Continued)

Figure Page

LIST OF TABLES

Table Page

CHAPTER 1
GENERAL INTRODUCTION

Grass seed production in Oregon and Willamette Valley

Oregon is the major global producer of cool-season forage and turf grass seeds (Young and Silberstein 2012). The Willamette Valley of Oregon produces nearly two-thirds of the cool-season grass seed in the United States (Anonymous 2016). The wet winters and dry summers of the Willamette Valley provide ideal growing conditions for grass seed production. Grasses grown in Oregon include annual ryegrass (*Lolium multiflorum* L.), bentgrasses (*Agrostis* spp.), fine fescues (*Festuca* spp.), Kentucky bluegrass (*Poa pratensis* L.), orchardgrass (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinacea* Schreb.) (Brewer 2005). Proper field management is a key to ensure the high quality standard of Oregon's seed for worldwide distribution.

Weed control is an important management practice both when establishing and in established stands of grasses grown for seed production. Weed competition can result in reduced seed yields and seed quality (Fairey and Lefkovitch 1999, Albeke et al. 1983). Furthermore, weed seeds can contaminate harvested grass seed, which increases the difficulty of cleaning seed to meet certification requirements (Horton et al. 2016). Grass weeds in grass seed crops are more difficult to control, because of their similar growth habits and seed features (Horton et al. 2016). Weed management in perennial grass seed crops, such as tall fescue and perennial ryegrass, can be more difficult than in annual grass crops, because the crops are in the field for multiple years. Ideally, weed

management should be applied before planting to ensure that fields are as clean as possible, but in established perennial crops, options become limited.

Poa trivialis is a weed problem in waterlogged perennial grass seed crop fields

Roughstalk bluegrass (*Poa trivialis* L.) is a cool-season perennial grass species which mostly spreads via seeds, but does have short stolons. This species grows well in wet conditions, but lacks heat and drought tolerance (Haggar 1979). Roughstalk bluegrass is often used in the southern US for overseeding in dormant warm season grass pastures and golf courses during winter.

Roughstalk bluegrass also is a weed in cool-season home lawns and golf courses. It forms irregular spots in pastures during summer. There are very limited control methods, either the chemical or cultural, for this weed species in mixed stand conditions. In grass seed crops, roughstalk bluegrass can compete for nutrients and contaminate the harvested grass seed (Haggar 1979). Roughstalk bluegrass seed are very small, with about 5.5 million per kilogram (Kaatz 2016). The small size and sticky nature of roughstalk bluegrass seed increases the difficulty and cost of seed cleaning.

This weed species has increased very quickly in the Willamette Valley, and in the past decade, complaints by growers about roughstalk bluegrass as a weed also have increased. The invasion of roughstalk bluegrass in grass seed crops, such as tall fescue and perennial ryegrass, often occurs in waterlogged soils. In these areas, roughstalk bluegrass exhibited better competitiveness and had higher population densities than other species.

The number of studies on roughstalk bluegrass is very limited. Previous studies indicated that roughstalk bluegrass prefers shady, high moisture environments and was sensitive to high temperatures (Vartha 1973, Rutledge et al. 2012b). The biomass, shoot and turf quality of roughstalk bluegrass was reduced more than other turf species, such as creeping bentgrass, at temperatures greater than 28 C (Budd 1970, Rutledge et al. 2012a). Furthermore, correlations were observed between the chemical components of roughstalk bluegrass including the decrease of total non-structural carbohydrate, proteins, fructan and amino acids concentrations as temperatures rose and moisture decreased (Budd 1970, Rutledge et al. 2012a, Rutledge et al. 2012b). However, results from previous studies do not explain the recent roughstalk bluegrass invasion in the Willamette Valley.

Competition and invasion influenced by waterlogging

Competition from weeds is one of the most important biological factors that reduces crop yield (Patterson 1995). Numerous studies have attempted to define the critical duration and factors of competition between weeds and crops for optimal weed management operations. Agronomic and environmental factors can either positively or negatively influence the competitive effects of weeds on crops. Environmental stresses potentially caused by factors such as unusual temperatures, flooding, drought, and soil degradation may impact weed/crop competition directly and interfere with weed management (Patterson 1995). Among these factors, soil conditions and temperature are two of the most important environmental factors that influence plant species competition (Mack et al. 2000).

Oxygen deficiency is the major environmental stress in a waterlogged soil. As a rate limited resource, the availability of soil oxygen is controlled by both oxygen diffusion from the atmosphere and the oxygen consumption by plants and soil microorganisms (Jury and Horton 2004, Cardoso et al. 2014). Waterlogging causes oxygen limitation in the rooting zone, because water reduces the gas diffusion flux (Jury and Horton 2004). Waterlogging can be found in some poorly drained fields after heavy rain or uneven irrigation.

Previous studies reported that the waterlogging or flooding may benefit some invasive species in wetland systems. In wetlands, species composition influenced by flooding was due to the variation of anoxia tolerance among the species (Fraser and Karnezis 2005, Mack et al. 2000). Invasion of non-native species in wetlands was successful because of better anoxia tolerance or increased photosynthesis during short-term flooding (Fraser and Karnezis 2005, Waring and Maricle 2012).

In contrast, influence of waterlogging on bio-invasion in a dryland ecosystem is not well understood. However, many studies have shown variation of waterlogging tolerance among dryland species. Because the physiological tolerance to abiotic stress may help the success of invasion (Fraser and Karnezis 2005, Waring and Maricle 2012), it is possible that waterlogging contributes to the roughstalk bluegrass problem in grass seed crop fields.

Physiological responses of plants to waterlogging and oxygen deficiency

Oxygen status of cells and tissues. The internal oxygen concentration in plants is controlled by the rate of internal oxygen diffusion. Internal diffusion is the most important mechanism of oxygen transport in plants (Wegner 2010, Geigenberger 2003). An oxygen gradient is required to drive the oxygen from the surrounding environment into the tissues and mitochondria where aerobic respiration occurs (Wegner 2010). The oxygen gradient needs to be steep enough to maintain a suitable oxygen flux rate.

There are three terms often used to describe the oxygen status of cells and tissues (Fukao and Bailey-Serres 2004). Normoxic is used to describe the cell state in an environment with normal oxygen levels or normoxia conditions. Anoxic is used to describe the cell state under anaerobic conditions or anoxia. Anoxia can be found in a soil environment with long term waterlogging. Hypoxic is used to describe the cell under the conditions between normoxic and anoxic. Hypoxic cells can be found under hypoxia conditions such as soils that receive heavy rainfall in a short time.

The oxygen state of root cells is likely to be heterozygous in a field whether the soil is well drained or waterlogged. In the waterlogged field, oxygen supply can be provided by downward gas transport from the aboveground parts of the plant when the soil oxygen level is low. In wetland species such as rice (*Oryza sativa* L.), tissue barriers are formed to prevent radial oxygen loss (ROL) from the roots (Colmer et al. 1998). On the other hand, hypoxic cells also can be found under normoxia conditions because of high metabolic activity (Geigenberger 2003). For the roots in waterlogged soils, anoxic

cells can be found deep within the tissues, surrounded by hypoxic or aerobic tissue (Colmer et al. 1998, Fukao and Bailey-Serres 2004).

Waterlogging influences on plant growth. Waterlogging may influence overall growth performance of plants (Waddington and Baker 1965, Wang and Jiang 2007). Stomata of the plants are likely to be closed at the early stage of waterlogging due to the high soil water potential (Pezeshki et al. 1996, Pezeshki 2001). Photosynthesis and gas exchange rate will be reduced due to stomata closure (Pezeshki et al. 1996). With the development of internal hypoxia, waterlogging stress will eventually trigger adverse effects on plant growth, such as reduced synthesis of total carbohydrate, proteins, lipid, amino acids, and chlorophyll (Drew, 1997, Phukan et al. 2014). Chlorophyll content of creeping bentgrass decreased when the water level raised from 15 cm to 1 cm below the soil surface (Jiang and Wang 2006). Previous studies indicated that the oxygen deficiency can inhibit amino acids synthesis via similar stress conditions caused by acetolactate synthase and 5-enolpyruvylshikimate-3-phosphate synthase herbicides which also inhibit amino acids synthesis (Zabalza and Royuela 2014).

For plants growing in waterlogged soils, the roots are the plant part most likely to be influenced by soil oxygen deficiency. Previous studies indicated that the biomass and elongation of the plant roots were limited in waterlogged soils (Drew 1997). Root tips are usually more sensitive and more likely to be injured by oxygen limitation compared to mature parts of the roots (Armstrong 1979, Wang and Jiang 2007). When waterlogging occurs in the seedbed, it mostly affects germinating seeds and young seedlings (Waters et al. 1991a, Waters et al. 1991b). Even though any further plant

development requires oxygen, some species such as rice (Straeten et al. 2001) and barnyardgrass (*Echinochloa crus-galli* L.) may germinate under anoxic environments (Fuko et al. 2003).

Cellular damages caused by oxygen deficiency. Though plant cells are able to survive anoxia for a short time, irreversible damage to mitochondrial structure, energy metabolism and cell viability happens within 15 hr under anoxic conditions (Andreev et al. 1991). Tissue damage and cell death arise principally because of the combined effects of reduced energy supply and self-poisoning with cytoplasmic acidosis during anaerobic metabolism.

Living plants cells generate ATP mainly via the glycolysis pathway. Reserved ATP in living plant cells can maintain the normal rate of metabolism for only 1 to 2 min once ATP generation stops (Drew 1997, Fukao and Bailey-Serres 2004). The shortage of oxygen in the tissues causes plant cells to switch from aerobic respiration to fermentation (Drew 1997, Fukao and Bailey-Serres 2004). There are three major types of fermentation in plant cells during oxygen deficiency: ethanol, lactic acid, and alanine pathways (Dennis et al. 2000). However, the amount of energy-provided to the cell is limited during hypoxia and anoxia conditions. The fermentation of one glucose molecule can only produce 2 ATPs, whereas the aerobic respiration of the same molecule can produce up to 38 ATPs (Geigenberger 2003). The lower energy generating level also may reduce the transportation of the sugar in phloem to roots, because the sugar unloading step may be influenced by the energy limitation (Saglio 1985, Waters et al. 1991a). The reduced sugar transportation most likely influences the apical zone of anoxic roots, even though

there are high concentrations of sugar and fructan in other parts of the roots (Waters et al. 1991b).

Self-poisoning in cells includes cytoplasmic acidosis and post anoxia stress. Cytoplasmic acidosis is regarded as the most important cause of cellular death under anoxic conditions (Drew 1997, Geigenberger 2003). Dramatic cytoplasmic pH decreases have been observed in the roots and aboveground tissues of rice, wheat (*Triticum* spp.) or maize (*Zea mays*) shortly after switching from normoxia to anoxia (Roberts et al. 1984, Gout et al. 2001). Cytoplasmic acidosis is mainly caused by the accumulation of toxic fermentation metabolites in the cytoplasm. The initial pH decrease may be caused by the accumulation of lactic acid, followed by acidification involving several other metabolic steps such as ATP hydrolysis (Drew 1997, Gout et al. 2001, Geigenberger 2003). Furthermore, because the molecular-oxygen serves as the terminal electron acceptor, oxygen re-entry during the post-anoxia phase results in the formation of chemically reactive oxygen species (ROS) (Biemelt et al. 1998). The accumulation of these toxic oxidative products may cause rapid peroxidative cell damage (Biemelt et al. 1998, Crawford and Braendle 1996).

Numerous studies have focused on the single mechanisms of cellular damage described above. During *in vitro* studies, seedling roots fed with hexose survived longer and fermented more actively under anoxia, but eventually died (Webb and Armstrong 1983). This result indicated substrate-starved arrest of glycolysis was not the only reason for cell death. The accumulation of lactic acid does not necessarily cause cell death either. *Limonium* species are tolerant to waterlogging and can maintain a high lactic acid

fermentation rate (Rivoal and Hanson 1993). Ethanol is the major toxic metabolite from ethanol fermentation, but has never been observed to reach a fatal concentration in plant cells (Drew 1997). Thus, cellular injury is likely to be caused by the combined effects of several mechanisms.

Waterlogging tolerance in plants

Although the absence of oxygen is usually fatal to plants, tolerance mechanisms may help plants to survive oxygen limited environments from hours to days or even longer if conditions permit (Kozlowski 1984, Geigenberger 2003). Once the plants fall into a low internal oxygen condition, the oxygen deficiency can be sensed by signal systems in the plants, which lead to adaptive responses that allow plants to survive or avoid internal anoxia (Geigenberger 2003). These responses vary with time-scale and levels of oxygen deficiency. But all these adaptive responses that help plants survive the oxygen limitation can be classified as either metabolic adaptions or morphology adaptions (Vartapetian and Jackson 1997). These mechanisms allow plants to use oxygen more efficiently, or increase internal oxygen diffusion to obtain more oxygen.

Metabolic adaptations. Metabolic adaptions to oxygen limitation are important, because maintenance of ATP generation and avoidance of cytoplasmic acidosis are essential for cells such as root apices to survive oxygen deficiency (Armstrong and Drew 2002). The metabolic adaptations usually cause changes to the overall protein component (Drew 1997, Armstrong and Drew 2002). Under oxygen deficiency, the normal protein synthesis pattern is changed by increasing the transcription and translation of some

selective proteins which are called anaerobic proteins (ANPs) (Xu et al. 2014). Anaerobic proteins, which include a set of enzymes, may enhance the performance of plants in low oxygen environments (Armstrong and Drew 2002, Xu et al. 2014).

There are three enzyme groups among these ANPs, which are most important to the fermentation and maintenance of metabolism during oxygen deficiency (Kennedy et al. 1992, Hanhijarvi and Fagerstedt 1995). Alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) are two groups of dehydrogenase enzymes that occur in plants and promote the alcohol pathway with the reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH), which may be limited during oxygen deficiency. ADH in grass species may increase during oxygen deficiency conditions (Bertrand et al., 2001). In a previous study of ADH activity in the roots of several marsh species, roughstalk bluegrass in waterlogged soil was used as a reference (Smith et al. 1986). This study found that the ADH activity of roughstalk bluegrass was greater in waterlogged soils, but it was insensitive to oxygen reduction compared to other tested marsh species (Smith et al. 1986). Lactate dehydrogenase (LDH) is an enzyme found in animals, plants, and prokaryotes which catalyzes the conversion between pyruvate and lactate (Drew 1997). However compared to ADH, LDH is believed to have a minor role in regulating the fermentation pathway. Overexpression of LDH activity in transgenic tomato did not increase the rate of the fermentation pathway (Rivoal and Hanson 1994).

Morphological and anatomical adaptations. Plants can adapt morphologically to gas oxygen deprivation during waterlogging. Morphological changes in some parts of the plant, especially the root, may help the plant either obtain or deliver oxygen more efficient. Fine roots usually have larger surface area to volume ratio than thicker roots.

This increased surface area can help increase the root soil oxygen efficiency (Silver et al. 2005, Ahmadi et al. 2013). The portion of surface roots in the roots system of maize and sunflower (*Helianthus annuus* L.) increased in response to waterlogging (Jackson et al. 1981, Jackson 1983). These surface roots benefit the plant by utilizing the oxygen from the upper soil layer, which may have richer oxygen than deeper layers (Jackson 1983). It has been reported that the number of lateral roots developed from an aerenchymatous root axis might be reduced during waterlogging (Armstrong et al. 1983, Sorrell et al. 2000, Cardoso et al. 2014). The advantage of reducing lateral roots under oxygen shortage is that the oxygen consumption by lateral roots is reduced, which increases the oxygen diffusion to the elongation zone of the root (Sorrell et al. 2000, Cardoso et al. 2014). During waterlogging, the proportion of roots of the tropical forage grass *Brachiaria humidicolar* increased in the upper soil layers (Cardoso et al. 2014). Another important structure that helps roots to survive waterlogged soil is root aerenchyma (Armstrong 1979, He et al. 1994). The formation of aerenchyma in low oxygen stressed plants is also known as the anatomical adaptation (Takahashi et al. 2014). Aerenchyma is a specialized tissue that forms in some species under hypoxic conditions that facilitates gas exchange with the root system. Aerenchyma formation is induced by programed death of cells in the root cortex, which increases root porosity (Maricle and Lee, 2002). Furthermore, the forming of lysigenous aerenchyma not only transfers extra gas to the root system, but also reduces the oxygen demand by reducing oxygen consuming tissues (Maricle and Lee, 2002).

Roughstalk bluegrass management and research objectives

All environmental factors that influence weed/crop competition will influence weed management strategies (Patterson 1995). The central hypothesis of this study is that the increasing roughstalk bluegrass populations in waterlogged seed crop fields is due to better waterlogging tolerance of roughstalk bluegrass, which is based on either changes in metabolism, morphology, or both. Potential practical recommendations for improving roughstalk bluegrass control in grass crop fields may be developed with a better understanding of the physiological responses of roughstalk bluegrass to waterlogging.

Thus, the research objectives of this study were to conduct physiological and morphological examinations of roughstalk bluegrass responses to waterlogging stress, and to detect potential mechanisms that contribute to the roughstalk bluegrass invasion in cool season grass seed crop fields, under oxygen deficient conditions.

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CHAPTER 2

INFLUENCE OF OXYGEN DEFICIENCY ON ROUGHSTALK BLUEGRASS AND TALL FESCUE DURING GERMINATION AND EARLY ESTABLISHMENT

ABSTRACT

Roughstalk bluegrass is an emerging weed problem in grass seed fields of the Willamette Valley of Oregon, especially in waterlogged fields. Two germination studies were conducted to a) detect the influence of oxygen deficiency and temperature, and b) the effects of waterlogging on seed germination of roughstalk bluegrass (RB) and tall fescue (TF). The first determined that oxygen deficiency resulted in a germination delay in both species, but the delay for TF was greater than for RB. Alcohol dehydrogenase (ADH) activity in both RB and TF seedlings increased under anoxic treatment. The increased ADH activity in both anoxic treated RB and TF seedlings reached the greatest levels at 8 h after treatment, and lasted longer in RB seedlings. Higher temperatures (20 - 30 C) increased the influence of oxygen deficiency on TF, but did not influence the effect of oxygen deficiency on RB. The second study determined that in compared to TF, the influence of waterlogging and oxygen deficiency treatment on RB was less during seed germination, but greater during the early establishment stage. Twenty eight days of waterlogging during the early establishment stage reduced the aboveground biomass of RB and TF by 58 and 46%, respectively, but did not influence seedling numbers. These features may help RB get a quicker establishment during seed germination stage, and

slow down the accumulation of toxic fermentation metabolite during early establishment stage, which is a major mechanism of plant damage during waterlogging.

INTRODUCTION

The competition between weed and crop may start as early as seed germination, because the germination stage is important for biological development of the plant (Holst et al. 2007, Nadem et al. 2013). The weed seedbank is the reserve of viable weed seeds present on the soil surface and in the soil profile (Benoit et al. 1989, Buhler et al. 1997). The weed seedbank consists of both new weed seeds recently shed and older seeds that have persisted in the soil, which may influence weed management for several years. Biological advantages including rapid germination and early establishment may contribute to the competitive success of invasive species (Bohumil 2003). Selection during crop breeding produces crops with a high germination percentage immediately after sowing in the uniform conditions of fields prepared for planting. In contrast, natural selection over a range of ecological conditions has resulted in different strategies in germination behavior among weeds (Benoit et al. 1989). Thus, weeds may be more competitive when environmental stresses are present.

Roughstalk bluegrass (*Poa trivialis* L.) has become a frequent grass weed problem in desirable turfgrass lawns and seed production fields. It is characterized by a light green leaf coloration and extensive stoloniferous lateral stems that result in irregular patches. Roughstalk bluegrass can be invasive in lawns or grass crop fields if seeds or stolons are present in the soil (Budd 1970). It is necessary to reduce roughstalk bluegrass in seed crop fields in order to produce quality turf-grass seed. In the Willamette Valley of Oregon, roughstalk bluegrass was observed to reach germination peaks during early November, late February and March. High temperatures before late October, low

temperatures during late November to February or low precipitation from April to October may limit germination. Seedlings of roughstalk bluegrass overwinter in the vegetative state, and produce seed in late spring and early summer.

Oxygen shortage is the primary environmental stress for plants in waterlogged soil due to limited gas diffusion (Drew 1997). Even though oxygen is necessary for seedling development (Fukao and Bailey-Serres 2004, Wang et al. 2013), germination under oxygen deficient conditions varies among species. Germination under anoxic conditions has been observed in some species including rice (*Oryza sativa* L.) (Alpi and Beevers 1983), barnyard grass (*Echinochloa crus-galli* L.) (Kennedy et al. 1980), coast coral tree (*Erythrina caffra* L.) (Small et al. 1989), and silk floss tree (*Chorisia speciose* L.) (Joly and Crawford 1983). In contrast, some species such as African locust bean (*Parkia pendula* L.) (Scarano and Crawford 1992), stop germinating when the oxygen concentration is low, and this response may help the seedling avoid cell damage from seasonal flooding (Kolb and Joly 2010).

Temperature is another important factor that affects seed germination (Basso 2007). Determining the temperature response of seeds is an important process in order to understand seed ecology and to predict seed germination (Roberts 1988). Roughstalk bluegrass seed start germinating when soil temperature is greater than 10 C (Budd 1970). But, higher temperatures do not always benefit seed germination. A study on the related species, annual bluegrass (*Poa annua.* L), indicated that the germination of eight annual bluegrass ecotypes varied from 1 to 23% at 39 day/29 C night, from 49 to 89% germination at 29 day/19 C night, and from 76 to 94% germination at 19 day/10 C night

(McElroy et al. 2004). According to the weather data archives from the Hyslop Research Farm, Corvallis, OR (44° 38' 03"-123° 11' 24"), the temperature range in the Willamette Valley during November, February, and March is between 5 C and 27 C. Based on these observations, the optimum germination temperature for roughstalk bluegrass is likely below 30 C.

Metabolism in anaerobic stressed plants may turn to a fermentation pathway to maintain energy production. The main product of fermentation in germinating seeds is ethanol (Ray et al. 2016). Alcohol dehydrogenase (ADH) is an enzyme family associated with alcohol fermentation, which can be induced by low oxygen stress (Fukao and Bailey-Serres 2004). Increased ADH will promote the production of the ATP necessary for the survival of the cell. Increased ADH activities have been observed in germinating seed of several species under oxygen deficiency (Magneschi and Perata 2009, Kolb and Joly 2010, Estioko et al. 2014). An example of ADH activity increasing is in submerged rice coleoptiles (Saika et al. 2006, Magneschi and Perata 2009). ADH activity increases during anoxic rice germination is essential for rice coleoptile elongation and tolerance to oxygen deficiency. Quick elongation of shoots can potentially reduce the complete submerce time of rice seedlings. Once the shoot breaks the water surface, the improved internal oxygen transport from the re-emerged shoots to submerged parts may reduce the oxygen deficiency (Magneschi and Perata 2009).

Weed-crop competition during plant establishment has been linked to reductions in the establishment and yield of tall fescue (*Festuca arundinacea*) (Charles et al. 1991, McElroy and Breeden 2006). Germination of roughstalk bluegrass seed may occur in

establishing or established tall fescue fields. Often, the number of seeds which germinate is greater than the number of seedlings that survive through the establishment stages (Hager et al. 2015). Biotic or abiotic condition changes, such as weed management, may limit the establishment performance of both weed and crops, and alter the competitive relationships (Richmond et al. 2005, Larson et al. 2016). Information of waterlogging influences on seedling establishment is lacking for either roughstalk bluegrass or tall fescue. However, a previous study indicated that the species diversity of grass communities was influenced when waterlogging occurred during germination and early establishment phases (Kotowski et al. 2010).

The objectives of this study were to test the influences of waterlogging on the germination and early establishment of roughstalk bluegrass and tall fescue, and to evaluate the influence of oxygen deficiency on the metabolism of roughstalk bluegrass and tall fescue seedlings.

MATERIALS AND METHODS

Plant material. A commercial RB cultivar (Quasar), and a tall fescue (TF, *Festuca arundinacea* Schreb.) cultivar (Rebel XLR), were used in this study. The study was conducted in climate controlled growth chambers. Seeds of both species were placed in petri dishes containing a moistened blotter paper. Each petri dish contained 25 seeds of one species.

Seed germination study. The study was conducted as a randomized complete block design with four replications. There were two species, three different levels, and three different temperatures in this study. Three growth chambers with constant temperature settings of 10, 20 and 30 C were used. A 24 hour light environment was supplied by six fluorescent light tubes (32 W at 5000 K) in each chamber. Each chamber contained treatments with three different oxygen levels, so there were 9 treatments (3 oxygen levels × 3 temperatures). Four petri dishes of each species were used for each treatment, and the study was repeated. A total of 200 seeds per treatment were used for each of the 2 species.

Three oxygen levels were used. At the normoxia level, the oxygen concentration was equal to the concentration in the surrounding environment. The hypoxia level was achieved by filling the petri dishes with deionized (DI) water to reduce the oxygen diffusion. Cheese cloth was used to cover the seeds in order to keep them submerged. For the anoxia treatment, the petri dishes were put into a laboratory anaerobic container (BD GasPak™ EZ Anaerobe Container System Sachets with Indicator, Becton Dickinson and

Company, East Rutherford, NJ 07417). The container and gas pack provided an anaerobic environment by absorbing the oxygen in the container.

Based on a preliminary study, the germination data were recorded every 4 days after the treatments (DAT) started. Seeds were counted as germinated when the radicle was visible. Germination of each treatment was averaged from the four petri dishes. The speed of seed germination index (SG) was calculated as

$$SG = \frac{number\ of\ seedlings}{days\ of\ 1st\ count} + \cdots + \frac{number\ of\ seedlings}{days\ of\ the\ final\ count}$$

The oxygen limitation treatments were removed at the end of the tests (28 d), but the seeds were kept in the same chambers with the same temperatures. The accumulated germination were calculated again at 10 days after treatments were removed.

Early establishment study. This study was conducted in green as a complete randomly design with two species, and under two oxygen levels (waterlogging and control). The greenhouse environment was 25 /20 C day/night with ambient sunlight plus lights providing 14 h light above 25 mW cm^{-2} per day. Fifty seeds of each species were planted in a 25 × 25 × 6 cm plastic tray filled with commercial potting medium (Sunshine Mix 1 Potting Mix; Sun Gro Horticulture, Bellevue, WA). Waterlogging treatments were applied by placing the trays into water filled 32 × 58 × 12 cm clear plastic boxes, when average seedling emergence reached 50%. There were two trays of each species in each plastic box, and the water level were kept at the soil surface. Number of seedlings were counted every 4 days for 28 days. The aboveground biomass was harvested at 28 DAT. Harvested aboveground biomass was dried at 65 C for 48 hr. The percent dry weight of

the treated plants relative to the untreated plants was calculated. Four replications (total of 200 seeds) were used per species for each treatment, and the study was repeated.

Soil redox potential was measured weekly by reading the soil's voltage (Eh) with an oxidation-reduction potential probe (WD-35649-50, Oakton Instruments, Vernon Hills, Il 60061) inserted about 1cm below the soil surface.

ADH enzyme assay. This study was conducted in germination chamber as a complete randomly design with two species, and treatments (anaerobic and control). Seeds were germinated in petri dishes as described previously in a germinator at 25 C. Each petri dish contained 25 seeds of either TF or RB. After 2 weeks, 16 petri dishes of each species were randomly selected, and put into the anaerobic containers. Besides the anoxic treatment, another 16 dishes of each species were set in the same germinator but outside of the anaerobic containers as non-treated controls. Four dishes per treatment per species (16 dishes total) were used for ADH activity measurements at 8, 16, 24, and 32 hr after treatment. The study was repeated.

The protein extraction followed the methods described by Proels and Huckelhoven (2014). Approximately 150 mg fresh tissue including both coleoptiles and roots was ground with liquid nitrogen. The powder was mixed in a 2000 ul microtube with 1.2 ml 50 mM Tris buffer containing 5 mM $MgCl_2$, 10 mM sodium borate, 1mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulphonyl fluoride, and 5mM dithiothreitol. The extracts were immediately centrifuged for 10 min at 13,000 g at 4 C. To obtain clear aliquots, the centrifuge process was repeated at least once. The protein

concentrations were quantified with Bio-Rad Quick Start™ Bradford protein assay kit (Bio-Rad, Hercules, California 94547) following the instruction manual.

The ADH activity assay was based on the following reaction:

$$\text{Ethanol}+\beta\text{-NAD} \xleftrightarrow{\text{Alcohol Dehydrogenase}} \text{Acetaldehyde}+\beta\text{-NADH}$$

The specific ADH activity was calculated as units per mg crude protein. One unit of alcohol dehydrogenase activity can convert 1.0 nmole of β-NAD to β-NADH per minute at pH 8.0 at 25 C. The reaction velocity was determined based on the method of Proels and Huckelhoven (2014) which measured the changes of absorbance at 340 nm resulting from β-NADH concentration changes. In a 2 ml centrifuge tube, 1.5 ml reaction mix included 0.65 ml 50 mM pH 8.0 sodium phosphate buffer, 0.05 ml 95% (v/v) ethanol, 0.75 ml 15mM β-NAD solution, and 0.05 ml crude protein sample solution. The reagents were mixed by inversion and immediately read at A340 for ~ 6 minutes. The absorbance was measured in a multitier spectrophotometer (Versa MAX microplate reader with Soft MAX Pro, Molecular Devices, Sunnyvale, CA 94089) with the chamber temperature set at 25 C. β-NADH production was calculated using standard concentration-absorbance curves. The ADH activity was calculated as

$$\text{U/mg crude protein} = \frac{\text{units per ml crude extraction}}{\text{mg protein per ml crude extraction}}.$$

Data analysis. The data were analyzed with software R (R Development Team, http://www.r-project.org/). ANOVA tests were performed to evaluate the differences among the treatments. Means were separated with Duncan's multiple range test at probability lower than 0.05.

RESULTS

The ANOVA test was performed with statistical models including parameters for submerge, temperature, interaction between submerge and temperature, DAT and replication. There were no differences among the replications in neither germination nor early establishment studies, so the data from the same treatment were combined for analysis. The germination was influenced by both oxygen limitation treatment and the temperature. The germination of TF also was influenced by the interaction between the submerge treatment and temperature, but germination of RB was not. Seedling biomass and ADH activities in both TF and RB were influenced by waterlogging and oxygen limitation treatment, but the seedling numbers were not.

No seed of either species germinated under the anoxia treatment. However, seeds of both species germinated when submerged in DI water (Fig. 2.1, 2.2, 2.3). Seeds under the submerge treatment germinated slower compared to the seeds under normal oxygen levels (Table 2.1). The germination delays for TF were greater than for RB. As shown in the Table 2.1, the delays between control groups and waterlogged groups were greater in TF. The inhibition from the submerge treatment decreased with increasing DAT for both species (Fig. 2.1, 2.2, 2.3).

Lower temperatures slowed the germination rate of both TF and RB species. At 10 C, both TF and RB seeds started to germinate at 8 DAT, while germinated seeds were observed at 4 DAT in the 20 and 30 C treatments. The optimum germination temperature was 20 C for both TF and RB species, and both species reached their greatest germination at 16 DAT. Germination under all treatments peaked before 28 DAT. Accumulated

germination of RB at 28 DAT was less at 10 C and 30 C compared with the 20 C treatment. Accumulated germination of TF at 28 DAT was greater at 20 C compared to the 10 C treatment, but was not different from the treatments at 30 C. This result indicated that the RB germination was more sensitive to the high temperature than was TF.

After the oxygen limitation treatments were removed, germination of previously anoxia treated seeds increased (Table 2.2). The greatest recovery of germination was 76% for TF and 68% for RB at 20 C. However, no germination increases were observed from seeds in the submerged or control groups.

The Eh of waterlogged soil was reduced by 21% at 28 DAT. The waterlogging treatment applied at early establishment stage did not reduce the emerged seedling numbers of either TF or RB (Fig. 2.4). At the end of the study (28 DAT), the seedling numbers of TF were 91 and 92% of planted seed in the control and waterlogging treatment groups, respectively. The seedling numbers of RB were 91 and 90% of planted seed in the control and waterlogging treatment groups, respectively. The waterlogging treatment applied at early establishment stage reduced the aboveground biomass of both TF and RB, and the waterlogging caused reductions were different between the two species ($P<0.01$). Compared to the untreated control, waterlogging reduced the aboveground biomass of TF and RB by 46 and 58%, respectively (Fig. 2.5).

ADH activities in the anoxic seedlings of both TF and RB increased at all the sampling times (Fig. 2.6). Generally, the ADH activity in TF seedlings was greater than in the RB seedlings which received the same treatments. After receiving the anoxic

treatment, TF species had a quicker (16 hr) and greater ADH activity peak (0.0152 U/mg) than RB (24 hr and 0.011 U/mg, respectively). The greater ADH activity in RB lasted longer than in TF. The ADH activities in anoxic treated TF seedlings increased 103.4, 353, 148, and 163% at 8, 16, 24, and 32 hours after treatment, respectively. The ADH activities in anoxic treated RB seedlings increased 99, 135, 331, and 287% at 8, 16, 24, and 32 hours after treatment, respectively.

DISCUSSION

Seed germination is a process with high energy requirements, which may be provided by respiration either aerobically or anaerobically (Ray et al. 2016). Neither RB nor TF seeds germinated under the anoxia environment suggesting that oxygen is required during the germination of these two species. The major effect of the submerge treatment was lowering the oxygen concentration around the germinating seeds, by limiting the gas flux rates (Tamang and Takeshi 2015). In this study, low oxygen concentration delayed seed germination rather than reducing the total germination. Submerge treatment delayed the rate of germination of TF more than RB. Because seed germination under oxygen deficiency is regarded as a capacity of seed to survive submerged conditions (Angaji et al. 2010, Kretzschmar et al. 2015), the RB seed may be either more tolerant to oxygen deficiency or has a lower oxygen concentration requirement.

Though temperature may only influence the effects of oxygen deficiency on TF, this influence may also affect the competition between TF and RB. Because oxygen deficiency delays TF germination more at higher temperatures, the effect of waterlogging interacted with the temperature. It is possible that higher temperature could influence competition between TF and RB during waterlogging. The purpose of the treatment removal study was to evaluate the potential damage to seed viability caused by low oxygen concentration. Though the seeds of neither TF nor RB species germinated in anoxia conditions, viability was not reduced by the anoxic treatment.

In the greenhouse study, aboveground biomass production rate of both TF and RB seedlings was sensitive to soil waterlogging. The influence of waterlogging was greater on RB than on TF. At the end of the study (28 DAT), dry aboveground biomass of waterlogged TF plants was 54% of untreated controls, while the dry aboveground biomass of waterlogged RB plants was 42% of untreated controls. The results suggest that the TF seedlings were more active during waterlogging treatment, which agreed with the result from ADH assay. Previous studies indicated that the changes of ADH activity in seedlings often represents the metabolic strategies of a species to oxygen deficiency during early establishment (Dennis et al. 2000, Magneschi and Perata 2009). The slower ADH activity peak of RB seedlings may indicate lower energy demand and growth rate during germination and emergence. Reduced growth rate in environmental stressed plants may contribute to avoidance of stress caused damage (Vasilikiotis and Melis 1994). Roughstalk bluegrass is one of the species that can tolerate environmental stresses by reducing its physiological activities. Roughstalk bluegrass can quickly go dormant during drought and hot weather (Budd 1970, Rutledge et al. 2012). The reduced activity during oxygen deficiency helps the plant to reduce cytoplasm acidosis and the following cell damage (Kozlowski 1984, Drew 1997).

Anaerobic germination and waterlogging tolerance have been well studied in waterlogging tolerant (rice, barnyardgrass) and intolerant (maize, pea, *Pisum sativum* L.) species. However, the physiological mechanism of anaerobic germination and its contribution to the following plant development cannot be explained by one general theory for all species. Estioko et al. (2014) reported that the differences in response to flooding by germinating seeds may influence the competition between rice and

barnyardgrass, but these differences were influenced by the timing and level of the waterlogging. Though the oxygen deficiency influences on total germination and emergency were similar between these two species in this study, different responses to waterlogging were observed between these two species. Compared to TF species, the influence of waterlogging treatment was less on RB seed germination, but greater on seedling growth. It is likely that the germination of RB seed can occur under lower oxygen concentration, but the growth of RB seedlings is more sensitive to oxygen deficiency. One potential benefit of this strategy is that RB may start to establish in the waterlogged soil earlier that TF, with minimum the cell damage induced by anaerobic respiration. Therefore, when waterlogging occurs during seed germination, RB seedlings may dominate the waterlogged area quicker than TF.

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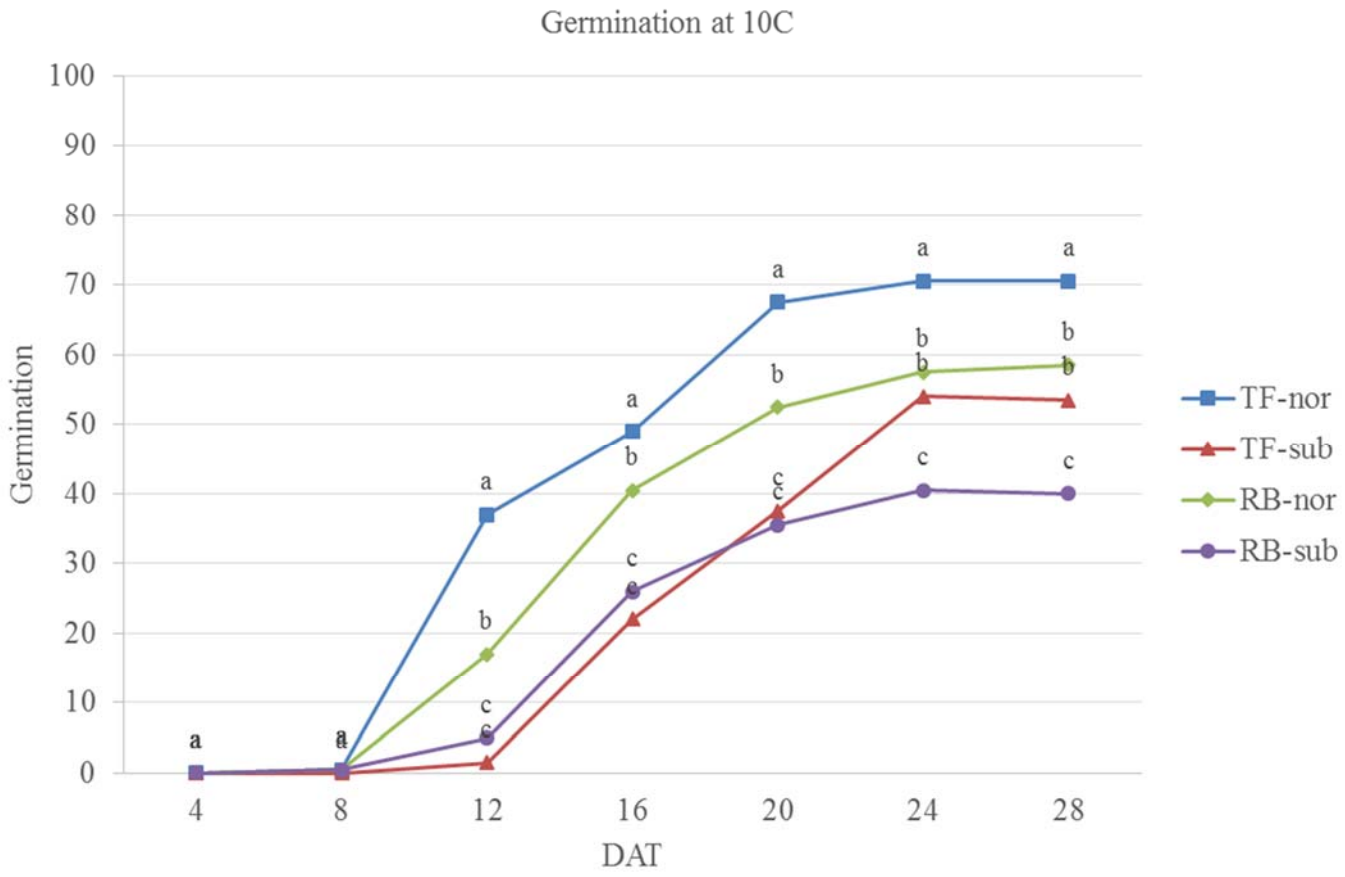

Fig. 2.1. Germination of RB and TF seeds at 10 C under two oxygen levels. nor = untreated control; sub = submerge treatment; DAT = days after treatment. Data represent the average of 8 replications, with 25 seeds per replication. Means with the same letter at the same measuring time are not different based on Duncan's multiple range test at 0.05 probability.

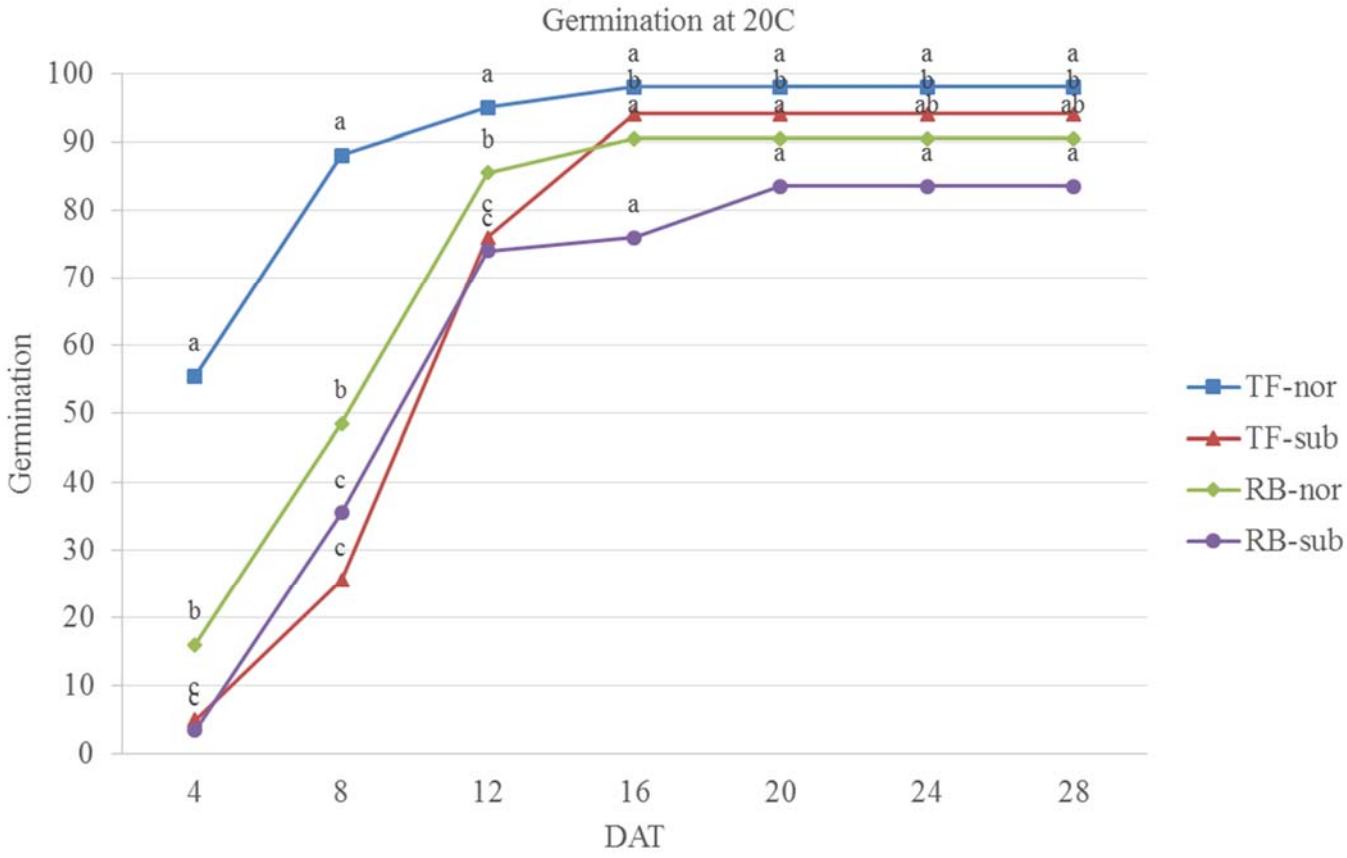

Fig. 2.2. Germination of RB and TF seeds at 20 C under two oxygen levels. nor = untreated control; sub = submerge treatment; DAT = days after treatment. Data represent the average of 8 replications, with 25 seeds per replication. Means with the same letter at the same measuring time are not different based on Duncan's multiple range test at 0.05 probability.

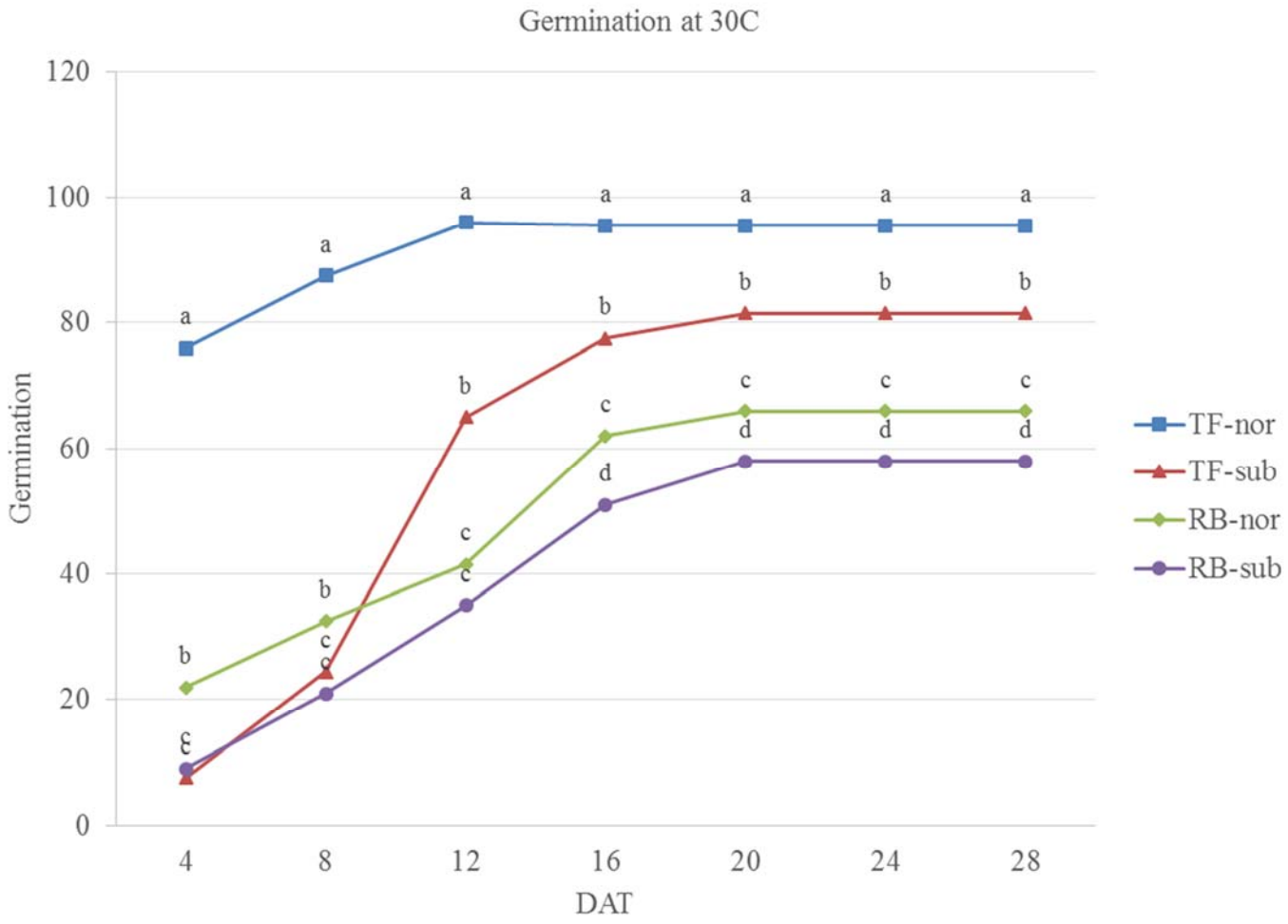

Fig. 2.3. Germination of RB and TF seeds at 30 C under two oxygen levels. nor = untreated control; sub = submerge treatment; DAT = days after treatment. Data represent the average of 8 replications, with 25 seeds per replication. Means with the same letter at the same measuring time are not different based on Duncan's multiple range test at 0.05 probability.

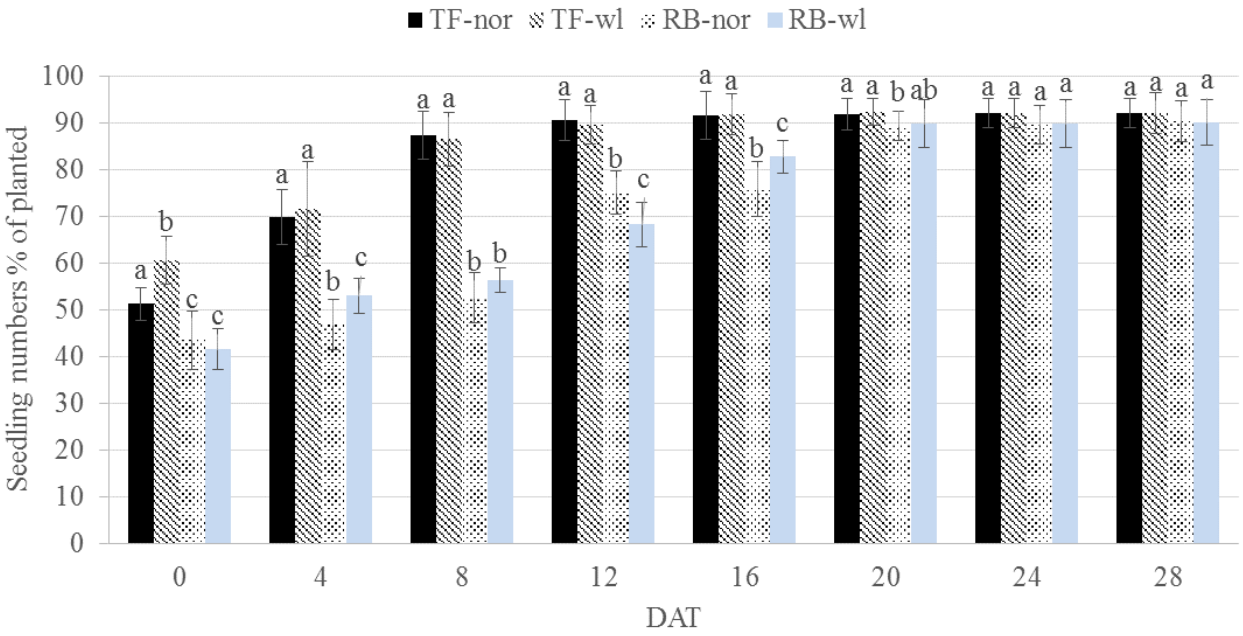

Fig. 2.4. Effects of waterlogging treatment during early establishment stage on seedling numbers. Seedling numbers were counted every 4 days for 28 days. nor= untreated control; wl= waterlogging treatment; DAT= days after treatment. Data represent the average of 8 replications, with 50 seedlings per each replication ± SD. Means with the same letter at the same measuring time are not different based on Duncan's multiple range test at 0.05 probability.

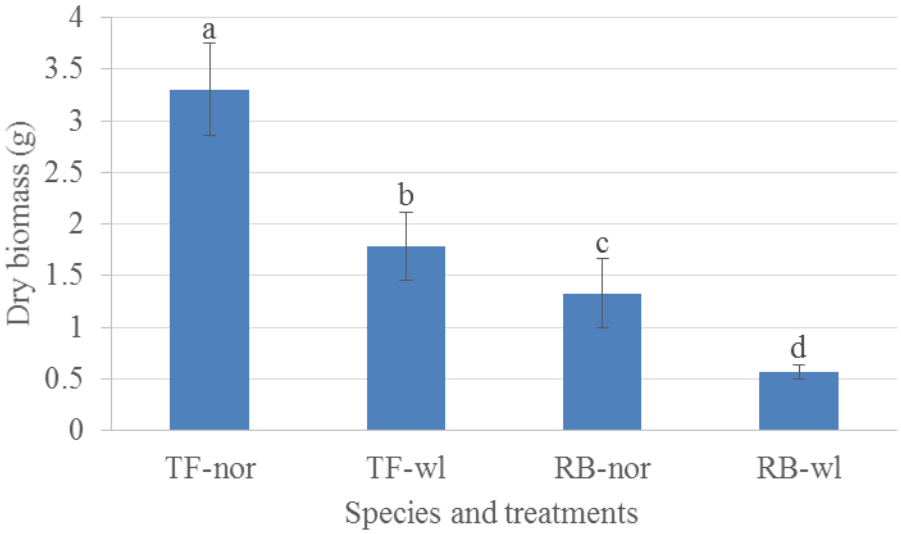

Fig. 2.5. Dry biomass of RB and TF seedlings after 28 days of waterlogging treatment during early establishment stage. Nor = untreated control, wl= waterlogging treatment. Data represent the average of 8 replications, with 50 seedlings per each replication ± SD. Means with the same letter are not different based on Fisher's LSD test at 0.05 probability.

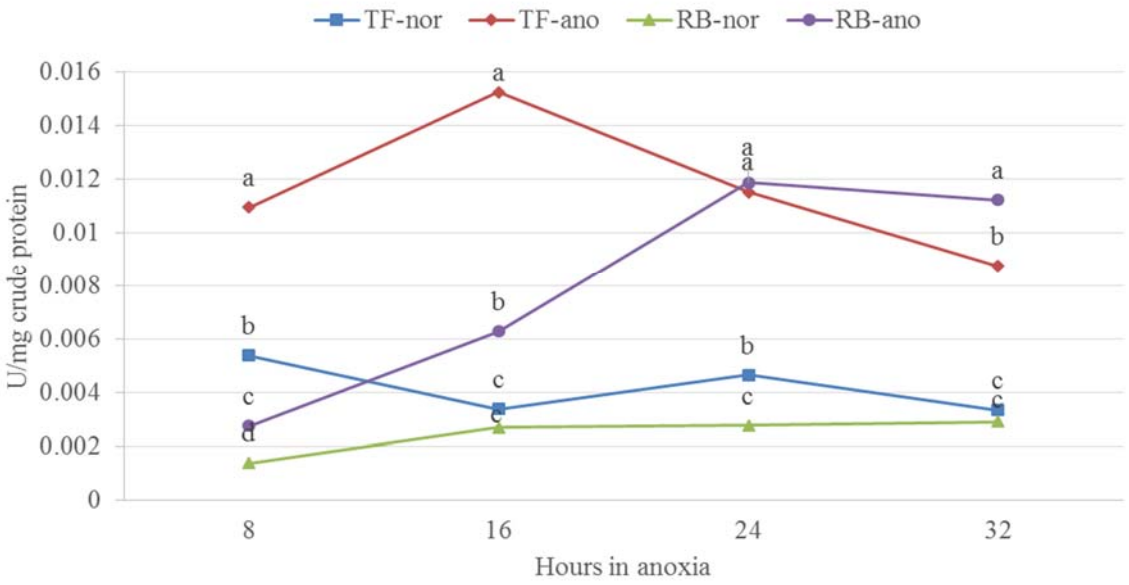

Fig. 2.6. Changes of ADH activities in anoxic treated tall fescue (TF) and roughstalk bluegrass (RB) seedlings. The specific ADH activity was calculated as units per mg crude protein. One unit of alcohol dehydrogenase activity (U) can convert 1.0 nmole of β-NAD to β-NADH per minute at pH 8.0 at 25 C. Data represent the means of 8 replications, with 25 seedlings per each replication ± SD. Nor = untreated control; ano = anoxic treatement. Means with the same letter in at the same measuring time are not different based on Duncan's multiple range test at 0.05 probability.

Table 2.1. Speed of germination index (SG) under different treatments. Data represent means of 8 replications, with 25 seeds per replication.

Species	Temperature (C)					
	10		20		30	
	nor	sub	nor	sub	nor	sub
TF	15aA	8bA	51cA	29dA	56cA	26dA
RB	11aB	7aA	34bB	27cA	25cB	18dB

[a]: nor = untreated control; sub=submerge treatment; ano= anoxia treatment.
[b]: Means followed by the same lower case letter in the row and upper case letter in the column are not different based on Fisher's LSD test at 0.05 probability.

Table 2.2. Accumulated germination (%) 10 d after treatment removal. Data represent the average of 8 replications, with 25 seeds per replication.

Species	Temperature								
	10			20			30		
	nor	sub	ano	nor	sub	ano	nor	sub	ano
TF	71a	60a	13a	98a	94a	76a	96a	82a	62a
RB	59b	42b	9a	91a	84a	67a	66b	58b	49b

[a]: nor= untreated control; sub=submerge treatment; ano= anoxia treatment.
[b]: Means followed by the same lower case letter in the row and upper case letter in the column are not different based on Student t-test at 0.05 probability.

CHAPTER 3

COMPARISON OF GROWTH AND PHYSIOLOGICAL CHARACTERISTICS BETWEEN ROUGHSTALK BLUEGRASS AND TALL FESCUE IN RESPONSE TO SIMULATED WATERLOGGING

ABSTRACT

Roughstalk bluegrass (*Poa trivialis* L.) is a weed problem in Oregon's cool season grass seed production fields. The population of this weed species in often greater in fields prone to waterlogging. A greenhouse study was conducted to investigate the morphological and physiological differences between recently established roughstalk bluegrass and a cool season grass seed crop tall fescue (*Festuca arundinacea* Schreb.) in response to waterlogging. Differences were found in root morphological development and root respiration between waterlogged tall fescue and roughstalk bluegrass plants. After 4 weeks of waterlogging treatment, the leaf number was reduced by 32 and 30% in tall fescue and roughstalk bluegrass, respectively. The plant height was reduced by 42 and 35% in tall fescue and roughstalk bluegrass, respectively. The root dry biomass was reduced by 43 and 13% in tall fescue and roughstalk bluegrass, respectively. The root length increased 6% in waterlogged tall fescue plants, and decreased 42% in waterlogged roughstalk bluegrass plants. In compared to tall fescues, the less reduced root biomass with decreased root length made roughstalk bluegrass root system shallower under waterlogging stress. At 1, 2, 3, and 4 weeks after treatment, root ADH activities increased 93, 45, 39, and 57% in waterlogged tall fescue, and increased 56, 27, 22, and 23% in waterlogged roughstalk bluegrass. At 1, 2, 3, and 4 weeks after treatment, root LDH activity increased 15, 18, 13, and 13% in waterlogged tall fescue, and 2, 19, 5, and 11%

in waterlogged roughstalk bluegrass. The turf quality, aboveground biomass, photosynthetic capacity, and water soluble carbohydrates concentration were reduced by waterlogging treatment, but the reductions were not different across timing or species. Thus, the shallower root system and the lower fermentation rate were the characters most likely to contribute to the better waterlogging tolerance in roughstalk bluegrass and invasion of roughstalk bluegrass in waterlogged cool season grass seed fields.

INTRODUCTION

Roughstalk bluegrass (*Poa trivialis* L.) is a weed species in cool season grass seed production fields. With very limited number of control options in Oregon's Willamette Valley, complaints about this weed problem from growers have increased in the past decade. Roughstalk bluegrass is often found in fields with waterlogging problems. It appears that waterlogging benefits roughstalk bluegrass in competition with other cool season grass species such as tall fescue and perennial ryegrass. However, the mechanisms underlying the relationship are not well understood.

Waterlogging is one of the major abiotic problems in some lowland areas (Jackson and Colmer 2005). Anoxia or hypoxia conditions may be found in waterlogged soils because air pores in saturated soils are filled by water and oxygen diffusion is blocked. Low oxygen concentration may influence a chain reaction including physical, chemical and biological processes in the soil (Pezeshki and DeLaune 1998, Wu et al. 2015). These processes may use oxidized compounds instead of oxygen molecules as electron acceptors, thus reducing soil redox potential (Eh, mV) (Pezeshki and DeLaune 2012). Soil redox potential is often used to describe waterlogging levels.

Waterlogging may eventually injure or even kill plants if it lasts long enough (Sairam et al. 2008). General injury symptoms for dryland plants under waterlogging stress include reduced photosynthesis, decreased growth of shoots and roots, decreased leaf numbers, leaf chlorosis and twisting, leaf abscission, root decay, and death (Cannel et al. 1979, Davies et al. 2000, Malik et al. 2002, Ashraf and Arfan 2005). However, several adaptive traits associated with waterlogging stressed plants may increase

waterlogging tolerance. Plant adaptations to oxygen deficiency may involve morphological and metabolic changes of above or submerged parts.

Morphological adaptation can help plants to mitigate oxygen deprivation during waterlogging or submergence (Jiang and Wang 2006). As reported in previous studies, flooding may result in existing root systems being replaced with new, morphologically distinct adventitious or lateral root systems (Hook 1984). Because oxygen concentration in the surface water or upper soil layers is greater than in deeper soil layers, adventitious and lateral roots can absorb oxygen more effectively from these oxygen rich areas. The alternative root systems have been observed in both dryland species (*e.g.* pea) and marsh plants (*e.g. Melaleuca* spp.) under flooding stress (Gomes and Kozlowski 1980, Armstrong et al. 1983, Florentine and Fox 2002). In addition to the changes in roots, morphological changes of aboveground parts also influence waterlogging tolerance in some species. A study conducted by Raskin and Kende (1984) indicated that the elongation rate of submerged rice shoots was faster than normal, which may reduce the submergence of rice seedlings. Increasing intercellular air spaces with aerenchyma formation is another type of important morphological or anatomical adaptation.

Photosynthetic light-response curves describe the net CO_2 assimilation of plant leaves as a function of photosynthetic photon flux density changes from dark to a high light level (Zhu et al. 2015). Photosynthetic light-response curves are commonly used to evaluate the photosynthesis performance under environmental stresses by describing the photosynthetic capacity, efficiency, and other parameters (Lobo et al. 2013). The photosynthetic light-response curves represent several photosynthetic traits including

dark respiration, light compensation point, photosynthesis-photosynthetic photon flux density relationship, and photosynthetic capacity (Markesteijn et al. 2007, Lobo et al. 2013). Other photosynthesis related parameters such as stomatal conductance and intercellular CO_2 are sensitive to photon flux density. Previous studies indicated that the stomatal conductance is positively related to the maximum photosynthetic capacity (Korner et al. 1979), while the intercellular CO_2 concentration has a correlation with their growing environment (Yoshie 1986, Waring and Maricle 2012). Reduced photosynthesis has been previously reported in many dryland species under waterlogging and concurrent salinity stresses (Naidoo and Mundree 1993). Because photosynthesis provides plants with required energy and carbohydrates, photosynthetic adaption is regarded as a major component of the waterlogging tolerance (Waring and Maricle 2012). Although high water potential induced stomata closure is regarded as the major reason for reduced photosynthesis during a short-term flooding (Pezeshki et al. 1996), photosynthetic pigment reduction has more influence during long-term waterlogging stress (Ou et al. 2011, Close and Davidson 2003).

Chlorophyll is one of the most important photosynthetic pigments in higher plants. Chlorophyll content reduction may eventually result in reduced photosynthetic capacity (Jimenez et al. 2015). Previous studies indicated that waterlogging treatments can reduce chlorophyll content at different levels in grass species (Ashraf and Arfan 1991, Jimenez et al. 2015). The effects of waterlogging on chlorophyll may also depend on the growth stage of the plant. For winter wheat, significant waterlogging induced reduction in chlorophyll content and photosynthesis may only happen at the tillering stage (Amri et al. 2014).

Metabolic responses to oxygen deficiency are essential for plants to survive waterlogging conditions, especially for cells from root apices (Greenway and Gibbs 2003, Fukao and Bailey-Serres 2004). One of the key processes for oxygen deficiency tolerance is to maintain necessary energy production with a limited oxygen supply, usually via modified anaerobic carbohydrate catabolism (Drew 1997, Fukao and Bailey-Serres 2004). As reviewed by Atwell et al. (1999), plant fermentation rate can be either slowed down or accelerated in response to quickly reduced oxygen concentration. Germinating lettuce seeds appear to slow down their anaerobic carbohydrate catabolism by 65% to survive short term anoxia (Raymond and Pradet 1980). In contrast, accelerated fermentation under oxygen deficiency has been observed in most species, such as in submerged rice coleoptiles (Huang et al. 2003, Magneschi et al. 2009). In some cases, fermentation may increase for the first 6 to 24 h and then turn to a much slower rate (Atwell et al. 1999). One benefit brought by increased fermentation is to maintain the energy production, which is important for supporting plant activity. However, increased fermentation does not always contribute to anoxia tolerance. Pea root tips may only survive anoxia for a very short time, even with an increased fermentation rate (Drew 1997, Atwell et al. 1999). Problems caused by increased fermentation rates include accumulation of toxic anaerobic metabolites and greater consumption rate of respiration substrates (Drew 1997). The toxic metabolite may cause cell injury and death, while a greater consumption rate of respiration substrates may cause the lack of substrates, eventually reducing energy production before oxygen level recovery (Drew 1997, Dennis et al. 2000). Thus, the regulation of fermentation rate is important to waterlogging tolerance of plants (Ricard et al. 1994, Ricard et al. 1998).

Alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) are two well-studied fermentation regulators, which are important to fermentation processes (Drew 1997, Wang et al. 2009). ADH are a group of dehydrogenase enzymes that regulate the interconversion between alcohols and aldehydes with the reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH). ADH is a parameter often used to quantify the rates of ethanol fermentation. ADH in grass species generally increases under oxygen deficiency conditions (Chung and Ferl 1999, Bertrand et al. 2001), but in roughstalk bluegrass, ADH was found to be less sensitive to waterlogging than other grass species (Smith et al. 1986). LDH is another important enzyme family found in animals, plants, and prokaryotes, which regulates the lactate pathway by catalyzing the conversion between pyruvate and lactate (Kelley 1989, Drew 1997). LDH is often used as a supplemental parameter in measuring fermentative activities of higher plants (Jackson and Herman 1982, Rivoal and Hanson 1994).

Water soluble carbohydrates (WSC) are the primary fermentation substrates in higher plant (Waite 1958, Downing et al. 2008). The type and concentration of WSC varies among species, growing environment, and growth stage of grasses (Saglio 1985). In temperate grasses, the primary forms of WSC are glucose, fructose, sucrose, and different types of fructans (Chatterton et al. 1989, Downing et al. 2008). In plants, WSC are the most essential component of plant nutrition that can be translated from a carbohydrate producer, or source (e.g. leaves, stems), to a carbohydrate consumer, or sink (e.g. roots, fruits) (Komor et al. 1977, Boorer et al. 1992). The concentration and distribution of WSC in cool season grass species vary depending on environmental condition and growth stage of the plants (Watts 2005). Thus, the WSC content of a given

plant organ is essentially dynamic with carbohydrate production, translation and consumption. For example, a high respiratory rate during hot weather may turn cool season grass leaves from a carbohydrate source to a carbohydrate sink (Watts 2005). WSC reserves may be reduced in waterlogged plant, because of the altered balance between photosynthesis and carbohydrate metabolism (Jurczyk et al. 2016). In many studies, waterlogging induced WSC changes are regarded as one of the relevant factors that influences the fermentation rate in some grass species (Jiang and Wang 2006, Wang and Jiang 2007, Manzur et al. 2009).

This study was conducted to compare the relative waterlogging tolerance between roughstalk bluegrass and tall fescue to investigate the physiological reasons that contribute to the spread of this weed species in the Willamette Valley. Thus, the specific objectives of this study were to evaluate the waterlogging influences on the morphology, metabolism, and photosynthesis of these two grass species.

MATERIALS AND METHODS

Plant material and general growing conditions. A commercial RB cultivar (Quasar), and a tall fescue (TF, *Festuca arundinacea* Schreb.) cultivar (Rebel XLR) were used in this study.

Studies were conducted in the greenhouse and laboratory at Oregon State University, Corvallis, OR. The study was a randomized complete block design with two treatments, waterlogging and control, and four replications. The greenhouse environment was 25 /20 C day/night with ambient sunlight plus grow lights providing 14 h light above 25 mW cm^{-2} per day. Seeds were germinated in petri dishes, and transplanted to pots in greenhouse when the coleoptiles reach 1.5 cm. Pots were 21 cm in length and 4.5 cm in diameter. Pots were filled with potting soil (Sunshine Mix 1 Potting Mix; Sun Gro Horticulture, Bellevue, WA) and placed into a 32 × 58 × 12 cm clear plastic containers. The waterlogging treatment was applied at 4 leaf stage. For the waterlogging treatment, the plastic containers was filled to the soil surface. In control groups, the water levels were kept at 18 cm below the soil surface. The water level was checked daily, water was added as necessary. The studies were repeated.

Soil redox potential. Rhizosphere redox potential was measured with an oxidation-reduction potential probe (WD-35649-50, Oakton Instruments, Vernon Hills, Il 60061). The probe was inserted to a 15 cm depth. The probe was connected and read using a benchtop pH meter (Accumet Research AR50, Fisher Scientific, Waltham, MA). The soil Eh was read at 1, 2, 3, and 4 weeks after treatment. At least two readings were made in each container.

Morphometric and biomass measurements. For the morphological and biomass measurements, turf quality, plant height (cm), leaf numbers, root length (length of the longest root, cm), aboveground dry biomass (g), and root dry biomass (g) were measured at 1, 2, 3, and 4 weeks after treatment. For each treatment, six plants will be randomly sampled from each plastic container (24 plants per species) at each measuring date. Turf quality was visually rated as an integral of color, shape and health on a scale from 0 (death, dry leaves) to 10 (healthy, green leaves). Aboveground biomass was harvested after plant height was measured and leaf number were counted. Roots were washed under tap water to remove soil, and root length was measured. The harvested leaves and roots were dried for 72 hr at 60 C and weighed. Vertical distribution of root biomass was measured, and was calculated as a ratio of gram root dry biomass per centimeter root depth (g DW cm^{-1}).

Photosynthesis and chlorophyll measurement. Photosynthetic response to waterlogging was evaluated via light response curve using a portable photosynthesis system (LI-6400XT, Li-Cor, Inc., Lincoln, NE, 68504). Net photosynthesis (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol CO_2 m^{-2} s^{-1}), and intercellular CO_2 concentration (Ci, μmol CO_2 mol air^{-1}) were read using system software (OPEN versions 6.2). At each designated measuring date, photosynthetic rate measurements were conducted between 10:00 and 15:00, when solar radiation was at maximum intensity. One healthy plant per replication was selected for the light response curve measurement. Two to three fully extended leaves from each selected plant were placed into the leaf chamber side by side with no overlap. The leaf area measured in the chamber was determined with a portable laser area meter (CI-202, CID Bio-Science, Camas, WA,

98607), and the photosynthetic data were adjusted using the corrected area. Light response curves were generated with a built in function of the system with seven radiant intensity levels at 1500, 1000, 500, 300, 150, 50, and 0 umol m^{-2} s^{-1}. The relative humidity in the chamber was 25%, with temperature of 25 C. The CO_2 concentration in the chamber was 360 ppm. Typically, 7 to 15 min was required for both photosynthesis and stomatal conductance to stabilize.

Following the photosynthetic measurement, fresh leaves were harvested for chlorophyll content estimation. Chlorophyll estimation was based on the method described by Vernon (1960). Four plants of each species were randomly selected from each replication (32 plants of each species). Approximately 100 mg (Wt1) of deveined leaf tissue from each plant were ground to a fine paste in 1 ml 80 % chilled acetone and the suspension centrifuged at 13,000 g for 15 min. Absorbance of the supernatant was measured at 645 and 663 nm in a multi titer spectrophotometer. Extinction coefficients used in this assay were 45.6 and 9.27 Lg^{-1} cm^{-1} for 645 and 663 nm, respectively (Inskeep and Bloom 1985). Amount of chlorophyll was calculated using the following formula:

$$\text{Total chlorophyll content in fresh leaf tissues (mg/g)} = \frac{A663 \times 8.02 + A645 \times 20.2}{0.3 \times Wt1}$$

The light response curve and total chlorophyll content were measured weekly at 1, 2, 3, and 4 weeks after treatment.

Metabolic responses to waterlogging. Metabolic responses of RB and TF to waterlogging were evaluated by measuring WSC content, ADH, and LDH activities at 1, 2, 3, and 4 weeks after treatment. Metabolic measurements were conducted in the

greenhouse and laboratory with a completely randomized design with four replications as described above. The study was repeated.

Crude protein were extracted following the methods described by Proels and Huckelhoven (2014). For each treatment, samples were randomly harvested from fresh leaves or roots of 4 plants per species. Harvested plant tissue was ground with liquid nitrogen. One hundred and fifty mg of the ground sample was extracted with 1.5 ml solvent contained 1.2 ml 50 mM Tris buffer containing 5 mM $MgCl_2$, 10 mM sodium borate, 1mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulphonyl fluoride, and 5mM dithiothreitol. Extract was purified by centrifuging at 13,000 g at 4 C for 10 min. The protein concentration was quantified with Bio-Rad Quick Start™ Bradford protein assay kit (Bio-Rad Laboratories, Hercules, CA) following the instruction manual.

ADH activity was determined based on the method described by Proels and Huckelhoven (2014). A 1.5 ml reaction mix included 0.65 ml 50 mM pH 8.0 sodium phosphate buffer, 0.05 ml 95% (v/v) ethanol, 0.75 ml 15 mM β-NAD solution, and 0.05 ml crude protein sample solution were mixed in a 2 ml centrifuge tube. The photons absorbance of sample solution was measured after the mixture was vibrated and incubated at 25 C for 5 min. The ADH activity was determined by measuring the increase of absorbance at 340 nm resulting from reduction of β-NAD using a multi-titer spectrophotometer (Versa MAX microplate reader with Soft MAX Pro, Molecular Devices) for 15 min. The specific ADH activity was calculated as units of ADH activity per mg crude protein (U/mg). One unit of ADH activity was defined as the amount that converts 1.0 nmole of β-NAD to β-NADH per minute at pH 8.0 and 25 C. The activity of

LDH was determined based on the method described by Wang et al. (2009). A 1.5ml reaction mixture containing 65 μl of extracted crude protein sample, 100 mM Tris–HCl (pH 8.0), 30 μM 4-bromopyrazole, 0.18 mM β-NADH, and 3.0 mM sodium pyruvate were mixed in a 2 ml centrifuge tube. The photons absorbance of sample solution was measured after the sodium pyruvate was added and vibration. The LDH activity was determined by measuring the decrease of absorbance at 340 nm resulting from the oxidation of β-NADH using a multi-titer spectrophotometer for 15 min. The specific LDH activity was calculated as units per mg crude protein (U/mg). One unit LDH activity oxidized 1 nmole β-NADH per minute at 25 C and pH 7.3.

The WSC extraction was designed based on the method described by Buysse and Merckx (1993) and Jensen et al. (2014). Leaf and root samples were harvested from four plants per treatment per species (32 plants of each species) at each measuring date. Samples were dried in a 60 C oven for 72 hr and ground in liquid nitrogen. Ten mg ground leaf or root tissue were put into a 2 ml centrifuge tube containing 1.5 ml deionized water, and boiled for 10 min. The tube was centrifuged at 13,000 g for 10 min, and the supernatant was collected. The extraction procedure was repeated. The supernatants of each plants were combined for WSC concentration analysis.

The extracted WSC concentration was quantified by the anthrone method described by Yemm and Willis (1954). Anthrone reagent was made by dissolving 1 g anthrone in 500 ml 72% sulphuric acid. One ml WSC extract and 5 ml ice-cold anthrone reagent was mixed in a 10 ml test tube. The mixture was heated for 11 minutes in a 100 C water bath and cooled rapidly to 0 C on ice. The mixture was read at 630 nm with a

multi-titer spectrophotometer. Concentration was determined by comparison to the standard curve.

Data analysis. Open source statistical software R (R Development Team, http://www.r-project.org/) was used to analyze the effects of waterlogging treatment. ANOVA test were performed to analyze the different responses to waterlogging between RB and TF. The means with different treatments were separated based on Duncan's multiple range test at P-value lower than 0.05.

RESULTS

Soil redox potential. The average soil Eh was reduced by 20, 36, 34 and 35% by waterlogging at 1, 2, 3 and 4 weeks after treatment (WAT), respectively (Table 3.1).

Morphometric and biomass measurements. All of the plants of both RB and TF survived the 4 week waterlogging treatment. Differences among data across replications or two studies were not significant, thus data with the same treatment were combined for analysis. All the measured parameters including turf quality, leaf number, plant height, aboveground dry biomass, root dry biomass, root length, and root distribution in both RB and TF were influenced by the waterlogging treatment (Table 3.2). There was not interaction between waterlogging and turf species on turf quality or aboveground biomass.

Turf quality and aboveground dry biomass of both TF and RB were reduced by the waterlogging treatment (Tables 3.3), but there was no difference between the species. At the end of the study (4 WAT), turf quality of waterlogging treated TF and RB plants were 54% and 57% of the untreated control, respectively. The major symptoms of waterlogging damage included yellowish and wilting leaves. Aboveground dry biomass of waterlogged TF and RB plants were 76 and 81% of the untreated control, respectively. Leaf number, plant height, root dry biomass, root length, and root distribution in waterlogging group were 68, 58, 57, 106, and 54% of untreated control for TF plants, and were 70, 65, 87, 58, and 150% of untreated control for RB plants (Table 3.3). Waterlogging treatment reduced leaf number and plant height more in TF than in RB. Waterlogging reduced root biomass in both RB and TF. The waterlogging treatment

reduced the root length in RB, but promoted the root length in TF. Under visual inspections, there was no significant waterlogging damage observed in the roots of either species. Compared with TF, the roots of RB were finer but were greater in numbers.

Photosynthesis and chlorophyll measurement. The maximum photosynthesis rates (A_{max}) of both TF and RB were reduced by waterlogging treatment ($P<0.05$) (Fig. 3.1). The reduction in photosynthesis rates was not relative to the duration of waterlogging. The *A* at 1500 umol m^{-2} s^{-1} (A_{1500}) of controlled TF ranged from 16.40 to 20.91 umol CO_2 m^{-2} s^{-1} during the four weeks study, with an average of 18.64 umol CO_2 m^{-2} s^{-1}. The average A_{1500} in waterlogged TF was reduced by 23%, and ranged from 13.01 to17.57 umol CO_2 m^{-2} s^{-1}. A_{1500} of controlled RB ranged from 13.62 to 17.88 umol CO_2 m^{-2} s^{-1} during the four weeks study, with an average of 15.73 umol CO_2 m^{-2} s^{-1}. The average A_{1500} in waterlogged RB was reduced by 35%, and ranged from 7.70 to11.87 umol CO_2 m^{-2} s^{-1}. The light compensation points were not different between control and waterlogging treated plants in either TF or RB. Saturation points were slightly reduced in both waterlogged TF and RB. The saturation point for normoxic TF and RB was around 500 mol m^{-2} s^{-1}, and for waterlogged TF and RB was around 300 mol m^{-2} s^{-1}.

Neither TF nor RB showed a difference in leaf stomatal conductance (*g*s) or leaf intercellular CO_2 concentration (*C*i) across treatments and treatment durations (Figs. 3.2-3.3). The *g*s at 1500 umol m^{-2} s^{-1} (gs_{1500}) ranged from 0.13 to 0.19 mol m^{-2} s^{-1} for TF, and from 0.11 to 0.18 mol m^{-2} s^{-1} for RB, respectively. The *C*i at 1500 umol m^{-2} s^{-1} (Ci_{1500}) ranged from 92.0 to 187.0 ppm for TF, and from 80.1 to 199.7 ppm for RB, respectively.

Significant decreases in chlorophyll concentration were observed in both waterlogged TF and RB compared to the control (Fig. 3.4). Chlorophyll concentration reduction increased with the duration of waterlogging ($P<0.05$). Significant reductions in chlorophyll concentrations in the waterlogged plants of both species were observed as early as two weeks after treatment. After 4 weeks of waterlogging treatment, chlorophyll concentrations were reduced by 23 and 25% in TF and RB, respectively, but were not different between the two species.

Metabolic responses to waterlogging. Waterlogging induced ADH activity changes were significant in roots but not in leaves (Fig. 3.5-3.6). In the leaves of waterlogged TF plants, the ADH activities increased 17, 2, 14, and 2% at 1, 2, 3, and 4 WAT. In waterlogged RB plants, ADH activities in leaves increased 12, 1, 8, and 1% at the same measuring dates. However, there were no differences between the two species. In contrast, ADH activities in waterlogged roots of TF were greater than in the roots of RB (Fig. 3.6). In roots of waterlogged TF, ADH activities increased 93, 45, 39, and 57% at 1, 2, 3, and 4 WAT. In roots of waterlogged RB, ADH activities increased 56, 27, 22, and 23% at 1, 2, 3, and 4 WAT.

Changes of LDH activity in waterlogged TF and RB were similar to the ADH activity, but were less sensitive. In the leaves of waterlogged plants, the LDH increased 6, 7, 3, and 3% in TF species, and 10, 1, 5, and 3% in RB species, at 1, 2, 3, and 4 WAT, respectively (Fig. 3.7). The leaf LDH activity was greater in TF than in RB. In the roots of waterlogged plants, significant increases in LDH activity were observed in TF through the entire study period, but not in RB. At 1, 2, 3, and 4 WAT, root LDH activity

increased 15, 18, 13, and 13% in TF species, and 2, 19, 5, and 11% in RB species (Fig. 3.8). The root LDH activity was greater in TF than in RB.

No significant leaf WSC concentration change under waterlogging condition was observed during the study, except in the leaves of RB plants at 2 weeks after treatment (Table 3.4). In contrast, waterlogging induced WSC concentration reductions were significant in the roots of both TF and RB. The root WSC concentration reductions ranged from 9.3 to 13.9% in TF and from 7 to 9% in RB.

DISCUSSION

During 4 weeks waterlogging treatment, significantly different responses were not observed in turf quality and dry aboveground biomass between TF and RB. However, variation in morphometric and metabolic responses between TF and RB species under waterlogging conditions indicated a potential diversity in waterlogging tolerance between these two species.

Among the morphometric and biomass responses, root development was the most distinguishable trait between two species in response to waterlogging treatment. Compared to TF, root distribution of RB was thicker and shallower in the soil. The thicker but shallower root system may help the roots of RB to absorb more oxygen from the upper soil layers. Furthermore, the diameters of RB roots are smaller than TF roots. With the same root biomass, RB has more roots in number than TF. Root systems with this feature usually have a greater surface area to volume ratio (Lamont 1983). This trait may also improve the waterlogging tolerance of RB species, because root systems with larger surface area to volume ratio usually have a higher efficiency of nutrient absorption and higher gas diffusion rates between root and soil (Lamont 1982, Guo et al. 2016). In contrast, the waterlogging induced reduction in aboveground dry biomass were not different between TF and RB. Thus, though leaf number and plant height showed differences between TF and RB in response to waterlogging, they should not be the major morphological reasons contribute to the RB invasion in waterlogged fields.

In both TF and RB plants, the maximum photosynthesis rates were reduced by the waterlogging treatments. However, the gas exchange characteristics including stomatal

conductance and intercellular CO_2 concentration in waterlogged plants were not different across treatments or treatment durations. Leaf chlorophyll content decreased in waterlogged TF and RB plants across the four treatment times. Chlorophyll is one of the most important photosynthetic pigments in higher plants, and the chlorophyll content is often influenced under environmental stress (Krause 1991, Huang et al. 1994, Parolin 2001).Thus, the reduction of chlorophyll content in waterlogged TF and RB plants may explain the reduction in photosynthetic capacity in these plants. Furthermore, according to the light response curves, the effective quantum yield (photosynthetic rate divided by absorbed irradiance before saturation point) were slightly less in waterlogged TF and RB plant. Because the effective quantum yield is proportional to efficiency of photosynthetic pigments for light capture (Ralph et al. 2002, Ralph and Gademann 2005), these changes give a support that the photosynthetic pigments were influenced by the waterlogging. In this study, some morphological responses, such as plant height and leaf numbers, were different between RB and TF. These differences may result in variation of photosynthetic production between these two species, because photosynthesis mainly takes place in leaves and may be influenced by factors such as leaf age, leaf position and leaf structure (Araus et al. 1986, Koike 1988, Kitajima et al. 2002). At the whole plant level, less reduced leaf number and plant height in waterlogged RB may eventually minimalize the influence of waterlogging in photosynthetic yield.

Adaptive response of root respiration is one of the most important factors that contributes to plant waterlogging tolerance (Drew 1997, Xu et al. 2014). In this study, root respiration was more sensitive to waterlogging than leaf in both TF and RB. Changes in alcohol and lactic acid fermentation usually represent adaptive strategies for

waterlogging tolerance (Xu et al. 2014). Increased ADH and LDH rates indicated oxygen deficiency occurred in waterlogged roots of both TF and RB. The lower ADH activities in waterlogged roots of RB plants indicated a lower fermentation rate compared with waterlogged TF plants. The reason that resulting for this lower fermentation was not detected in this study. It may due to better oxygen difussion as a result of the shallower root system, or because of lower metabolism activity under stress. Under the same conditions, lower fermentation activity produces less toxic metabolites, and causes less damage to the plant cells (Drew 1997, Fukao and Bailey-Serres 2004). Thus, RB plants may have less waterlogging induced cellular damages compared with TF plants. Previous studies indicated that starvation of respirable substrates may happen in roots, because sugars are unable to be delivered to the apical zone during waterlogging (Drew 1997). Though sugar concentrations are high at whole plant levels, sugars transportation in phloem can be reduced in waterlogged roots due to reduced energy production (Waters et al. 1991). For example, increased leaf WSC concentration and decreased root WSC were observed in the waterlogged Kentucky bluegrass plants in a previous study (Wang and Jiang 2007). However, according to the results of this study, the root respiration of these two species may not be influenced by lack of fermentation substrate. Though the root WSC concentrations were reduced in both waterlogged RB and TF plants, the reductions were not correlated with the duration of waterlogging treatment.

In summary, the main objective of this study was to determine if there were different physiological responses to oxygen deficiency between TF and RB. Waterlogging treatment in this greenhouse study created oxygen deficiency and lower redox potential conditions in the rhizosphere. Plants from both TF and RB survived the

four week long waterlogging treatment, but had similar reductions in growth rates, turf qualities, and photosynthetic capacities. Under waterlogging conditions, responses of root morphological development and root respiration were the most distinguishable traits between these two species. The shallower and thicker root system, and lower fermentation rate may help the roots of RB plants obtain more oxygen and reduce waterlogging induced damages. However, more research should be completed on the physiological responses of TF and RB to oxygen deficiency in order to understand the different waterlogging tolerances observed between these two species in the field.

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Table 3.1. Soil redox potential reading during experiment.

Duration	Eh (mV)[a]	
	nor	wl
1 week	341	272
2 week	330	212
3 week	388	257
4 week	353	228

[a]nor = no treatment control, wl = waterlogging treatment

Table 3.2. Mean squares from the analyses of variances for turf quality, leaf number, height, aboveground dry biomass, root dry biomass, root length, and root distribution under different duration of waterlogging treatments.

Duration	Sources	df	Turf quality	Leaf number	Height	Aboveground dry biomass	Root dry biomass	Root length	Root distribution
1 week	Waterlogging	1	9.57***	19.14	484.38***	0.05	0.29***	22.78	0.95
	Species	1	0.03	372.00***	130.01***	0.25***	0.07*	81.28**	9.86***
	Waterlogging×Species	1	0.07	30.52*	0.01	0.04	0.01	648.00***	0.05
	Residuals	28	0.55	6.18	5.68	0.02	0.01	8.13	0.37
2 weeks	Waterlogging	1	142.38***	82.88*	484.38***	0.11***	0.46***	23.63	0.17
	Species	1	3.13	331.53***	202.51***	0.42***	0.02	207.57***	4.03**
	Waterlogging×Species	1	1.76	0.20	0.78	0.05**	0.16*	599.35***	2.30*
	Residuals	28	2.48	14.08	7.54	0.01	0.02	7.71	0.33
3 weeks	Waterlogging	1	147.71***	250.32***	127.10***	0.04*	0.91***	91.10*	2.05*
	Species	1	1.42	234.61***	121.10**	1.76***	0.29**	354.91***	2.23*
	Waterlogging×Species	1	0.10	162.00***	59.81*	0.01	0.04*	1.10	6.03***
	Residuals	28	1.53	3.73	13.67	0.01	0.01	14.4	0.41
4 weeks	Waterlogging	1	132.03***	331.53***	128.14**	0.39*	0.74***	232.47**	7.48**
	Species	1	0.01	1505.63***	388.51***	2.89***	1.68***	1803.75***	4.42*
	Waterlogging×Species	1	0.19	42.78**	76.57*	0.07	1.01***	463.22***	4.98*
	Residuals	28	0.89	4.33	16.20	0.02	0.05	21.81	0.85

[a]P-value: *** < 0.001 < ** < 0.01 < * < 0.05

Table 3.3. Turf quality, leaf number, height, aboveground dry biomass, root dry biomass, root length, and root distribution of the two species under normal and waterlogging conditions.

Species	Treatment[a]	Turf quality	Leaf number	Height	Aboveground dry biomass	Root dry biomass	Root length	Root distribution
				cm	g	g	cm	g dw cm^{-1}
					Week 1			
TF	nor	9.72a	10.00a	26.09a	0.61a	0.68a	14.06a	0.048a
	wl	8.72b	10.41a	18.28b	0.46ab	0.53b	18.75b	0.021b
RB	nor	9.88a	18.78b	22.03c	0.36b	0.81c	19.88c	0.041c
	wl	8.69b	15.28c	14.28d	0.36b	0.58d	12.56d	0.046ac
					Week 2			
TF	nor	9.69a	12.25a	29.72a	0.70a	0.68a	16.94a	0.040a
	wl	5.00b	8.88b	21.63b	0.50b	0.58a	27.31b	0.021b
RB	nor	9.84a	18.53c	24.38c	0.39c	0.87b	20.50c	0.043a
	wl	6.09b	15.47d	16.91d	0.35c	0.49c	13.56d	0.036c
					Week 3			
TF	nor	9.25a	9.44a	33.00a	1.00a	1.02a	44.34a	0.023a
	wl	4.84b	8.34b	17.66b	0.93a	0.76b	41.34b	0.018a
RB	nor	9.56a	31.06c	26.38c	0.53b	0.91ab	23.66c	0.038b
	wl	5.38b	20.97d	16.50d	0.46c	0.50c	19.91d	0.025c
					Week 4			
TF	nor	9.25a	12.78a	37.47a	1.34a	1.53a	38.56a	0.040a
	wl	5.03b	8.66b	21.72b	1.02b	0.87b	40.78b	0.021b
RB	nor	9.13a	28.81c	27.41c	0.64c	0.77bc	31.16c	0.025bc
	wl	5.22b	20.06d	17.84d	0.52c	0.67c	18.16d	0.037a

[a]: nor = no treatment control, wl = waterlogging treatment.

[b]: Means in the same measuring time followed by the same letter in the column are not different based on Duncan's multiple range test at 0.05 probability.

Table 3.4. Leaf water soluble carbohydrate (LWSC) content and root water soluble carbohydrate (RWSC) content in tall fescue (TF) and roughstalk bluegrass (RB) during the 4 week greenhouse study. Data represent means of 32 individuals.

Population	Treatment[a]	LWSC (mg g^{-1} DW)			
		week1	week2	week3	week4
TF	nor	114.7aA	120.2aA	147.4bA	139.7bA
	wl	114.6aA	122.1bB	147.2cA	144.2cA
	Decreased (%)[b]	0.1	-1.6	0.1	-3.2
RB	nor	144.1aA	136.5bA	159.6cA	163.0cA
	wl	143.0aA	131.6bB	164.3cA	159.8cA
	Decreased (%)	0.8	3.5	-2.9	1.9
		RWSC (mg g^{-1} DW)			
TF	nor	104.8aA	101.2aA	107.7abA	114.7bA
	wl	90.2aB	91.9aB	97.7abB	103.2bB
	Decreased (%)	13.9	9.3	9.3	10.0
RB	nor	96.3aA	98.4aA	108.7bA	108.0bA
	wl	89.7aB	89.8aB	99.4bB	100.2bB
	Decreased (%)	6.9	8.7	8.6	7.2

[a] nor = no treatment control, wl = waterlogging treatment.

[b] percentage decrease in water soluble carbohydrate content for waterlogged plants, negative numbers represent increased percentage.

[c] Means followed by the same lower case letter in the row and upper case letter in the column are not different based on Duncan's multiple range test at 0.05 probability.

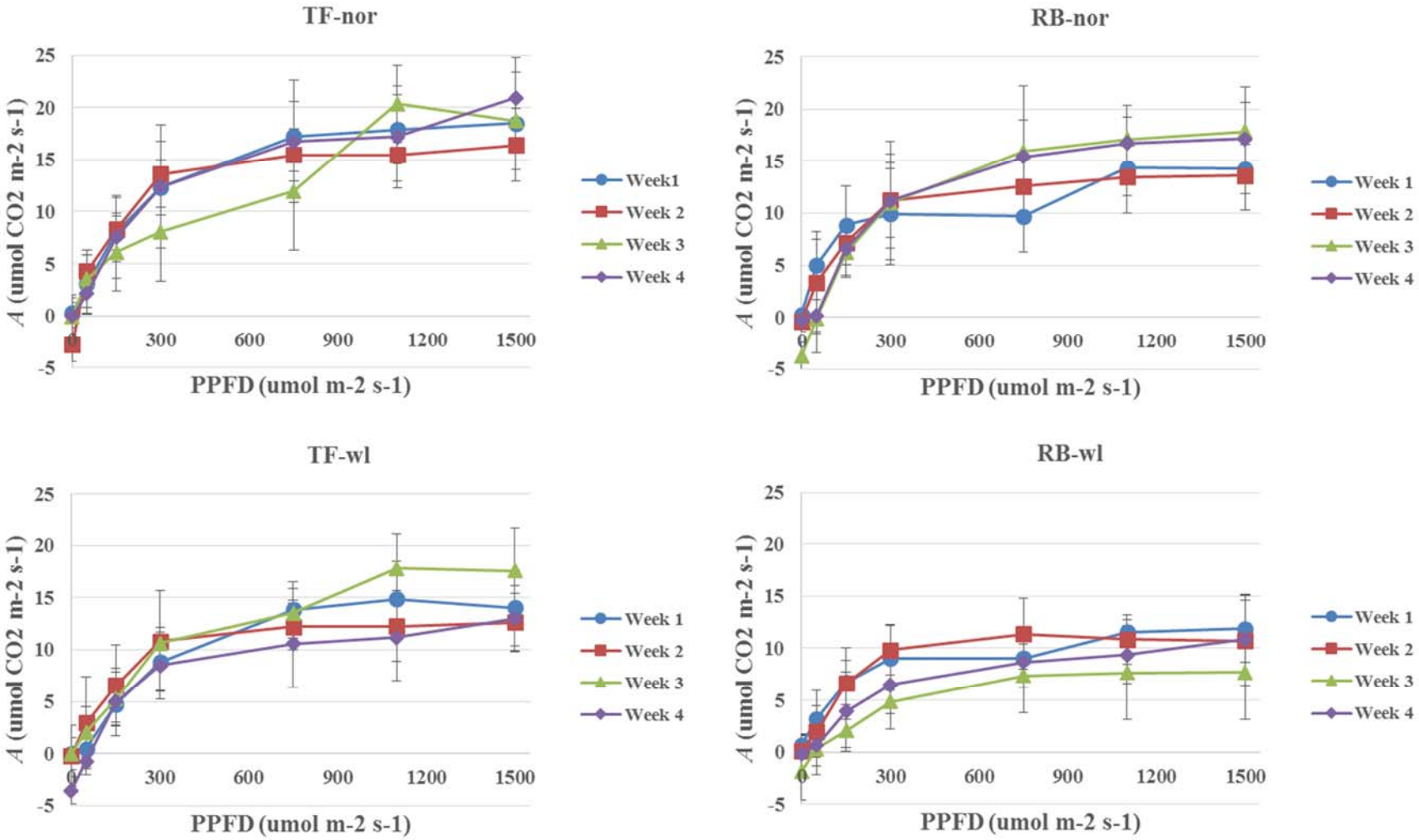

Fig. 3.1. Response curves of leaf net photosynthetic rate (*A*) as a function of photosynthetic photon flux density (PPFD) in normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means with ±SD of 8 individuals.

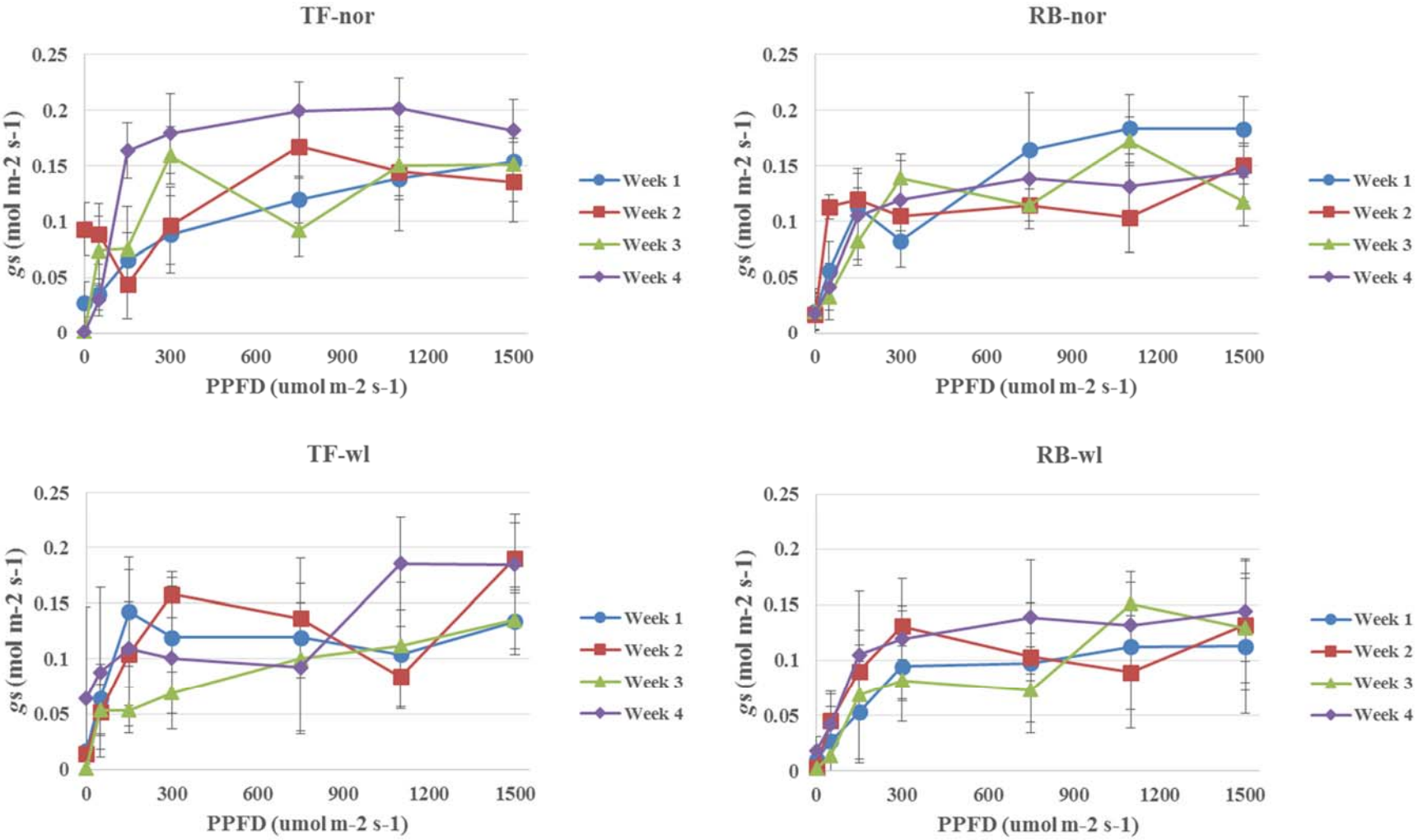

Fig. 3.2. Response curves of leaf stomatal conductance (*g*s) as a function of photosynthetic photon flux density (PPFD) in normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means with ±SD of 8 individuals.

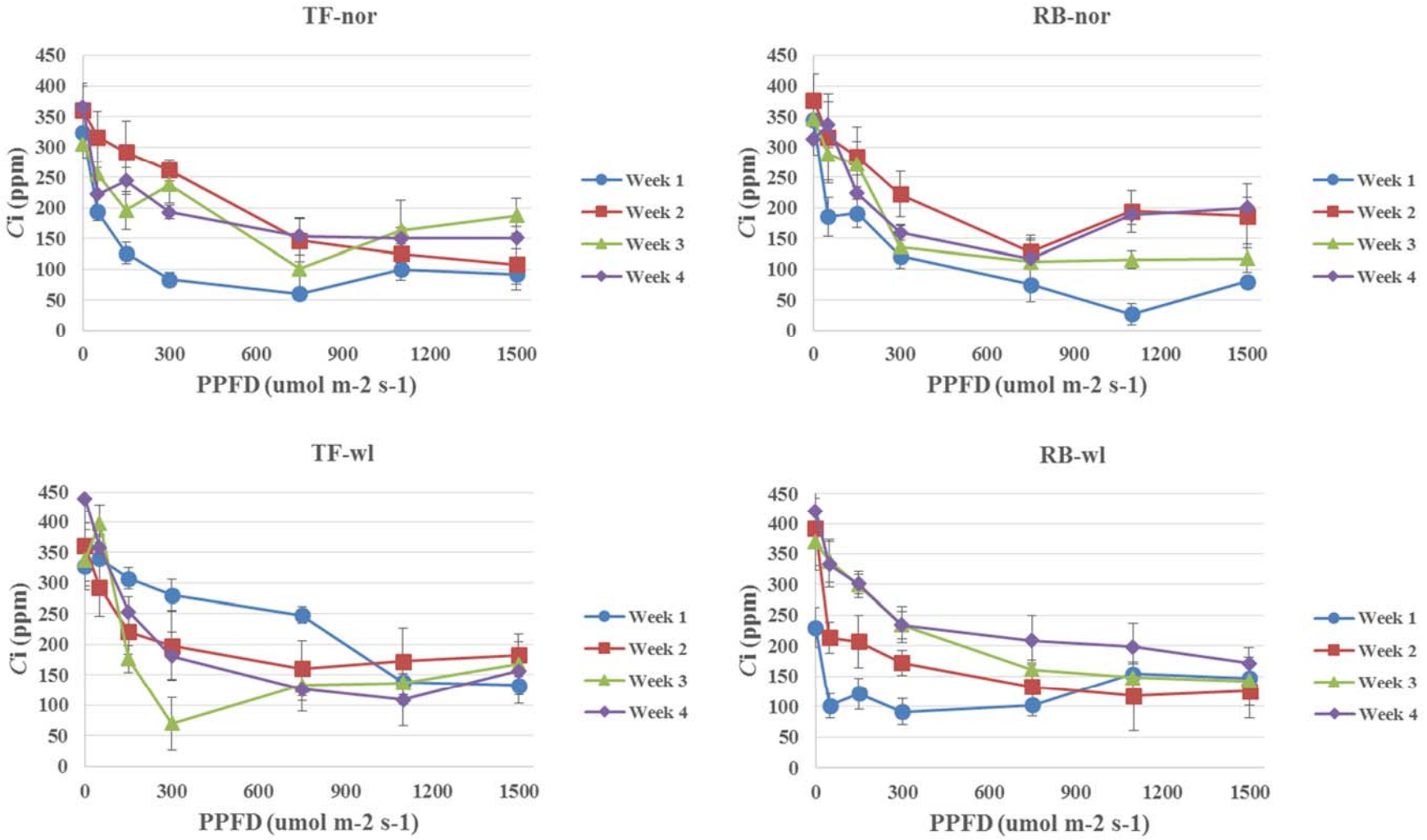

Fig. 3.3. Response curves of leaf intercellular CO_2 concentration (*Ci*) as a function of photosynthetic photon flux density (PPFD) in normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means with ±SD of 8 individuals.

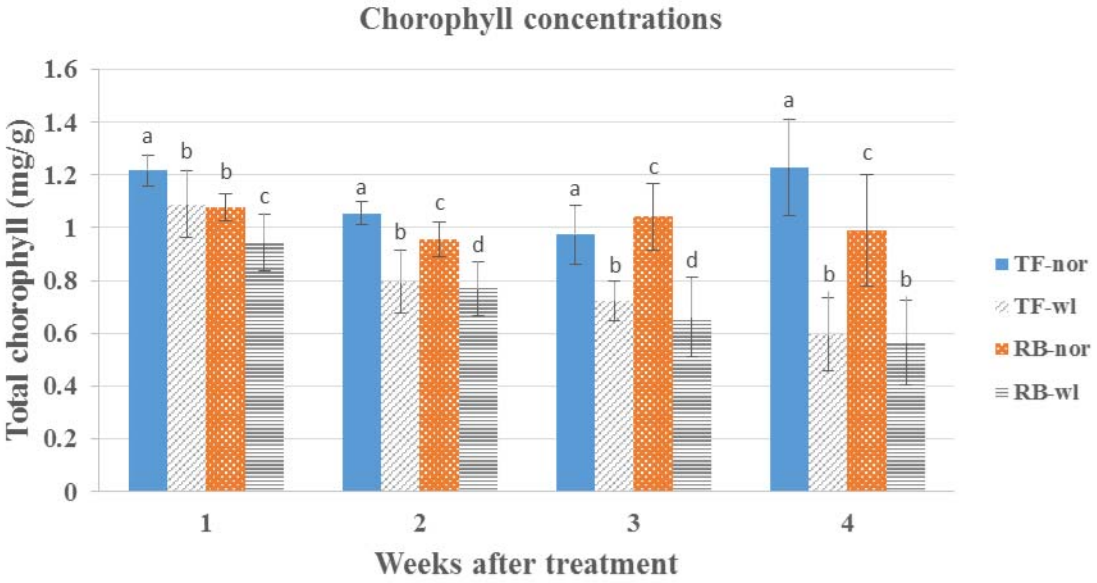

Fig. 3.4. Chlorophyll concentration in normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means with ±SD of 32 individuals. Means in the same measuring time followed by the same letter are not different based on Duncan's multiple range test at 0.05 probability.

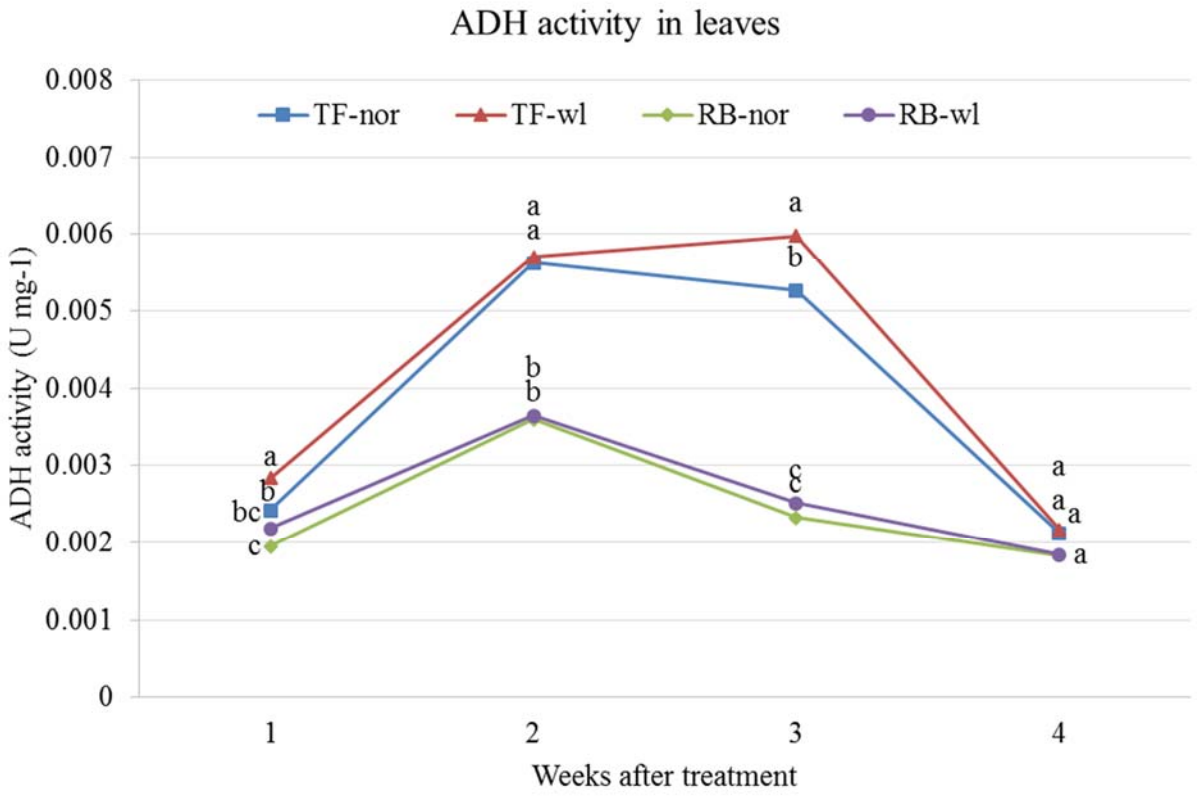

Fig. 3.5. ADH activity changes in leaves of normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means of 32 individuals. Means at the same measuring time with the same letter are not different based on Duncan's multiple range test at 0.05 probability.

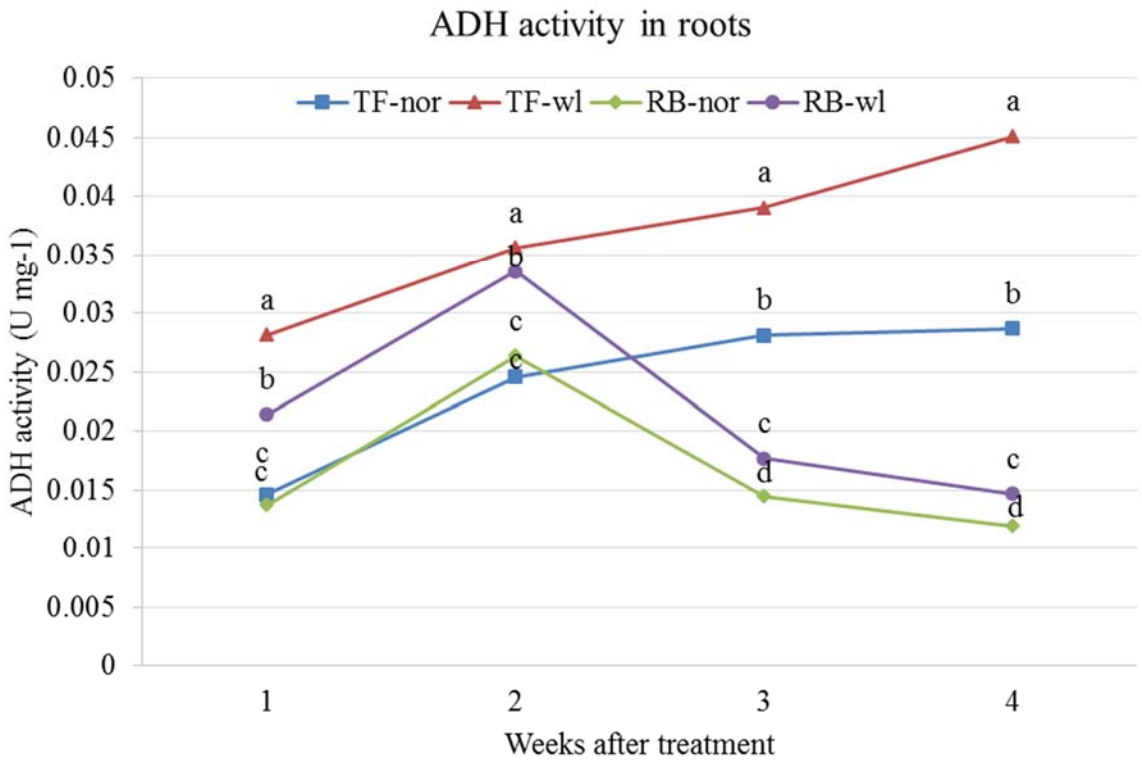

Fig. 3.6. ADH activity changes in roots of normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means of 32 individuals. Means at the same measuring time with the same letter are not different based on Duncan's multiple range test at 0.05 probability.

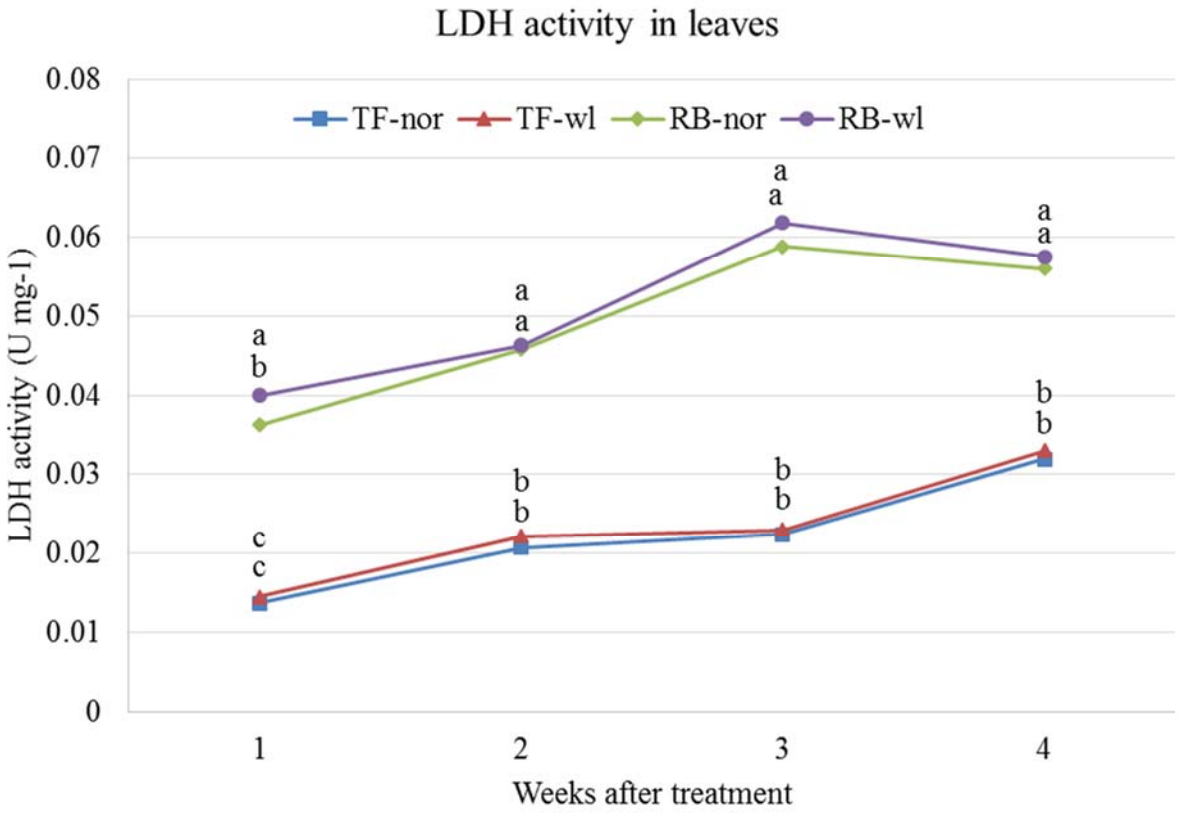

Fig. 3.7. LDH activity changes in leaves of normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means of 32 individuals. Means at the same measuring time with the same letter are not different based on Duncan's multiple range test at 0.05 probability.

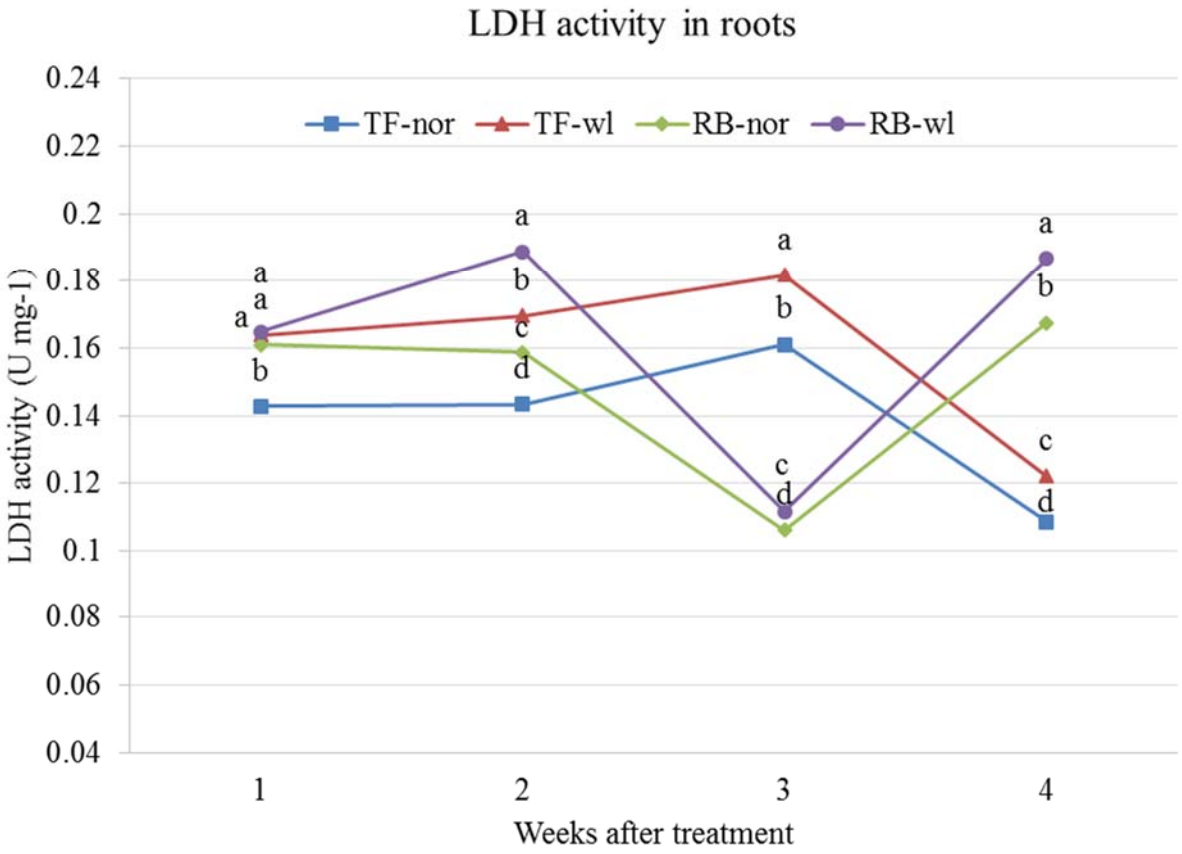

Fig. 3.8. LDH activity changes in roots of normoxic tall fescue (TF-nor), normoxic roughstalk bluegrass (RB-nor), waterlogged tall fescue (TF-wl), and waterlogged roughstalk bluegrass (RB-wl) during the 4 week greenhouse study. Data represent means of 32 individuals. Means at the same measuring time with the same letter are not different based on Duncan's multiple range test at 0.05 probability.

CHAPTER 4

AERENCHYMA FORMATION AND ROOT POROSITY CHANGES IN ROUGHSTALK BLUEGRASS AND TALL FESCUE DURING WATERLOGGING

ABSTRACT

Roughstalk bluegrass (*Poa trivialis* L.) (RB) is cool season grass species that may have the capacity to develop a root aerenchyma system. Root aerenchyma may contribute to better waterlogging tolerance by improving oxygen transport efficiency in waterlogged roots and reducing oxygen consumption. With these benefits, aerenchyma may be one of the physiological traits that contributes to the RB invasion in waterlogged cool season grass seed production fields. A greenhouse study was conducted to investigate the aerenchyma development and root porosity changes in RB and tall fescue (*Festuca arundinacea* Schreb.) (TF) under waterlogging conditions. After a four week waterlogging treatment, the aerenchyma by area portions were 23 and 34% in drained and waterlogged TF roots, and 29 and 38% in drained and waterlogged RB roots. After a four week waterlogging treatment, the root porosities were 25 and 31% in drained and waterlogged TF plants, and 23 and 30% in drained and waterlogged RB plants. The aerenchyma formations increased with the root porosities under the waterlogging conditions in both TF and RB. The greater aerenchyma portion and its contribution to root porosity increase may resulting better waterlogging tolerance in RB.

INTRODUCTION

Roughstalk bluegrass (*Poa trivialis* L.) (RB), is a cool-season perennial grass species found throughout the United States. In the Willamette Valley, RB is a grass weed species in grass seed production fields. RB can survive under many of the cool season turfgrass growing conditions and cultural weed control methods (McCullough and Hart 2011, Reicher et al. 2011). RB thrives in moist soils and high levels of shade. The spread of RB in desired turfgrass species can be reduced by decreasing irrigation frequency (Turf tips 2016). In grass seed crop fields of the Willamette Valley, RB is often found in areas with waterlogging problems. It is possible that the waterlogged soils benefit the invasion of RB in grass seed crop fields.

Most cool season grass species, including RB, are sensitive to waterlogging stress, but some species are more tolerant than others. The major reason of waterlogging induced plant damage is the hypoxia resulting from lower gas diffusion rate in water (Ferrel and Himmelblau 1967). When the oxygen concentration drops below a certain level, aerobic respiration is inhibited (Drew 1997, Armstrong et al. 2009). Though plants may switch aerobic respiration to anaerobic respiration at different levels, energy production is reduced during oxygen deficiency due to the low efficiency of anaerobic respiration (Hanhijarvi and Fagerstedt 1995, Geigenberger 2003). Furthermore, the accumulation of fermentation metabolites can damage the plant cell when it reaches a toxic level (Drew 1997).

Roots are the most vulnerable plant part during waterlogging stress. Oxygen that is consumed by root cells is provided by oxygen diffusion from air-filled cavities in the

soil (Armstrong 1979). The diffusion rate is not only controlled by the oxygen concentration in the soil, but also controlled by internal oxygen diffusion (Armstrong et al. 1982). The root apical zone with less intercellular spaces and low internal gas diffusion rate is sensitive to soil oxygen deficiency even in normoxic environment when temperature is high (Armstrong and Beckett 1985). Although several convective mechanisms of gas transport have been identified in plants, diffusion is still considered to be the major mechanism for oxygen transport towards the root apex in plants (Wegner 2010). As indicated by previous studies, the oxygen diffusion rate is primarily controlled by oxygen concentration gradient, pathway length, and diffusion resistance (Armstrong et al. 1983, Armstrong and Beckett 1987). For the root system, the oxygen concentration gradient can be influenced by the respiration and oxygen concentration in soil, while the length and the resistance of the oxygen transport pathway can be controlled by root properties (Armstrong and Beckett 1987).

Though the oxygen concentration is limited in waterlogged soil, some waterlogging tolerant plants can develop anatomical structures such as lacunae in the shoot and aerenchyma in the root to increase the internal gas diffusion rate for effective oxygen supply to those hypoxic organs (Wegner 2010). Previous studies indicated that the fractional porosity of the root is a very important parameter that influence the internal gas transport resistance (Armstrong 1979, Armstrong et al. 1983, Wegner 2010). The greater fractional volume that is taken by gas space in root may result in a lower gas transport resistance, because of greater oxygen diffusion rates in the air filled space (Armstrong et al. 1982). However, in a previous study, root porosity can only provide a rough measurement for the resistance of the root to gas transport, the anatomical

examination into the root structure is still necessary to investigate the plant's response in gas transport during oxygen deficiency (Wegner 2010).

Aerenchyma is a structure that forms in the leaves, stems and roots of some plants, which enhances internal aeration between or within shoots and roots (Colmer 2003, Takashashi et al. 2014). Depending on the position where the aerenchyma forms, aerenchyma can be divided into primary aerenchyma, which forms in primary tissues, and secondary aerenchyma, which forms in secondary tissues (Takashashi et al. 2014). There are two types of primary aerenchyma: schizogenous aerenchyma is formed by the separation of cortical cells or enlargement of existing intercellular space, while the lysigenous aerenchyma is formed by the collapse and lysis of cortical cells due to programmed cell death (Justin and Armstrong 1987, Armstrong and Armstrong 1994, Jackson and Armstrong 1999). Lysigenous aerenchyma is the major type of aerenchyma formed in many monocots, including cool season grass species (Armstrong and Armstrong 1994, Takashashi et al. 2014). Though the mechanism of lysigenous aerenchyma formation is not fully understood, most previous anatomical and physiological studies indicated that the formation of lysigenous aerenchyma may related to the ethylene accumulation in the hypoxic root of waterlogged plant (Drew et al. 1979, Evans 2003, Visser and Voesenek 2004).

The formation of aerenchyma in hypoxic roots under waterlogging condition is regarded as an important adaptation for survival of waterlogged plant (Armstrong 1979, Drew et al. 1981, Appleby 1984). In fully developed lysigenous aerenchyma, the lysed cortical cells of the root are digested, leaving only the cell walls along with the outer cell

layers and epidermis (Evans 2003). This structure increases the internal gas filled space, and therefore improves the gas diffusion efficiency between the environment and the endodermis (Armstrong 1979, Drew et al. 1979). This gas diffusion includes the oxygen diffusion from oxygen rich environment to hypoxic cells and delivering CO_2 produced by root respiration (Justin and Armstrong 1987, Constable and Longstreth 1994). A recent study conducted by Karahara et al. (2012) in rice roots indicated that some well-connected channels in a longitudinal direction along the root axis were formed by the remnants of lysed cells, which provide extra longitudinal pathways for internal root cells. Furthermore, because the roots with aerenchyma have fewer cells in the same volume due to the programed cell death, these roots consume less oxygen in comparison with the roots without aerenchyma (Drew 1997).

The objective of this study was to evaluate the different influences of waterlogging on the formation of lysigenous type aerenchyma and the changes of air-filled root porosity.

MATERIALS AND METHODS

Plant material and general growing conditions. A commercial RB cultivar (Quasar), and a tall fescue (TF, *Festuca arundinacea* Schreb.) cultivar (Rebel XLR) were used in this study.

The study was conducted as a randomized complete block design with four replications. Two treatments, control and waterlogging were used. Study was conducted in the greenhouse at Oregon State University, Corvallis, OR. The greenhouse environment was 25 /20 C day/night temperature with ambient lights providing 14 h light above 25 mW cm^{-2} per day. Seeds were germinated in petri dishes, and transplanted when the coleoptiles reach 1.5 cm into 21 cm in length by 4.5 cm in diameter pots in the greenhouse. Pots were filled with potting soil and placed into a 32 × 58 × 12 cm clear plastic container. The waterlogging treatment was applied at the 4 leaf stage by adding water to the plastic containers, and maintained at the soil surface during the study. In the control group, water was added to the plastic containers, and maintained at 18 cm below the soil surface. Rhizosphere redox potential was measured with an oxidation-reduction potential probe (WD-35649-50, Oakton Instruments, Vernon Hills, Il 60061). The probe was inserted 15 cm below the soil surface. The probe was connected to a benchtop pH meter (Accumet Research AR50, Fisher Scientific, Waltham, MA). The soil Eh was read at 1, 2, 3, and 4 weeks after treatment (WAT). At least two readings were made in each container. In total 4 waterlogging containers and 4 control containers were used in this study, each contained eight RB plants and eight TF plants. The study was repeated.

Root sampling and measuring. At 1, 2, 3, and 4 WAT, roots from 2 RB plants and 2 TF plants per container (8 per species) were harvested, and carefully rinsed by the tap water to remove soils. Root sections between 2 to 10 cm from the root tip were sampled and hand sectioned to a length of 1 cm. The root samples were examined using a scanning electron microscope (SEM) for aerenchyma formation and a pycnometer method for root porosity measurement.

For aerenchyma formation examination, root samples were washed with deionized water (d.i.) water, and cut to 10 mm sections. The selected root sections were placed in to fixative provided by OSU Electron Microscopy Facility (Oregon State University, Corvallis, OR 97331). After soaking in fixative for 8 to 24 hr, root samples were rinsed with sodium cacodglate at 0.1 M concentration three times for at least 10 min each time. The fixed root samples were sent to the OSU Electron Microscopy Facility for further preparation. The final prepared root samples were viewed by field emission scanning electron microscopy (FESEM) using an FEI QUANTA 600F environmental SEM with an energy-dispersive X-ray (EDX) attachment.

The image analysis and aerenchyma area quantification method were designed based on Maricle and Lee (2002). Digital images of root cross sections were analyzed with Photoshop CC 2014 software (Adobe, San Jose, CA 95113) to measure the percentage of root aerenchyma by area in the root cross section. In the digital images of root cross section (Fig. 4.1A), a new layer in Photoshop CC 2014 was created to cover the original image. On this new layer, painting tools were used to trace the root cross section area in black ink (Fig. 4.1B). The area of the root cross section was quantified by

reading the pixels of the new layer, after withdrawing the original image (Fig. 4.1C). The area of the aerenchyma was quantified by the same method (Fig. 4.1D). The percentage of aerenchyma in the cross section was calculated as the ratio of aerenchyma area over the cross section area. The percentage of aerenchyma of each treatment was the average readings from three to five randomly selected cross sections from 2 to 10 cm from the root tip.

The root porosity measurement using the pycnometer method was described by Noordwijk and Brouwer (1989). The pycnometer or specific gravity bottle is a device used to determine the density of liquid. This method is based on a comparison of the density of intact root tissue and the same tissue without air space. About 1 g of root sample was put into a pycnometer, and the pycnometer was filled with d.i. water and weighed (W_1). The root sample was taken out and the water on the roots was removed by centrifugation, then weighed (W_2). Next, the roots were grounded in a mortar to remove the air space in the root, and weighed in the water filled pycnometer again (W_3). Lastly, the pycnometer filled with water was weighed (W_4). The root porosity was calculated as:

$$P_{root} = \frac{100 \times (W3 - W1)}{W4 - W1 + W2}$$

Data analysis. Open source statistical software R (R Development Team, http://www.r-project.org/) was used to analyze the effects of the waterlogging treatment. ANOVA test was performed to determine the difference of waterlogging effects across treatments and species. Duncan's multiple range test was performed to analyze the effect of waterlogging on root porosity and aerenchyma formation in RB and TF. A significant difference was determined at a P-value at less than 0.05.

RESULTS

The soil redox potential was reduced by waterlogging treatments compared with the control group (Table 4.1). The average soil redox potential (Eh) reading during the 4 week study was 334 mV for the control group and 280 mV for the waterlogging treated group.

The data were not different among replications or studies, thus the data from the same species and treatment were combined for analysis. Waterlogging influenced the formation of root aerenchyma and root porosity (Table 4.2). The waterlogging effects interacted with the species at some of the measurement timings. After four weeks of waterlogging, there was an interaction with waterlogging effect on the formation of root aerenchyma, but not on root porosity.

Lysigenous type aerenchyma was observed in the root cortex of both RB and TF, and in both drained and waterlogged plants (Fig. 4.2 and 4.3). The average aerenchyma in root cross sections by area ranged from 14 to 34% in TF, and from 13 to 38% in RB (Table 4.3). Aerenchyma areas increased in waterlogged TF at 1, 2, 3, and 4 WAT. In waterlogged RB roots, aerenchyma areas increased in 2, 3, and 4 WAT. At the end of the study, the aerenchyma were 23 and 34% in drained and waterlogged TF roots, and were 29 and 38% in drained and waterlogged RB roots. The waterlogging induced aerenchyma increases were greater in RB than TF.

Root porosities ranged from 19 to 31% in TF, and ranged from 19 to 30% in RB (Table 4.4). Root porosities of TF and RB increased under both controlled and waterlogged conditions. In waterlogged TF plants, the root porosities were greater at 2

and 4 WAT. In waterlogged RB plants, the root porosities were greater at 2, 3, and 4 WAT. At the end of the study, the root porosities were 25 and 31% in drained and waterlogged TF plants, and were 23 and 30% in drained and waterlogged RB plants. Although the average waterlogging induced root porosity increase in RB was greater at 4 WAT (6% in TF versus 7% in RB), this difference was not significant.

DISCUSSION

Differences in the capacity of aerenchyma formation is regarded as one of the major factors that contributes to flooding tolerance of wetland species (Justin and Armstrong 1987, Takahashi et al. 2014). Many studies have been conducted with different species to determine the relationship between aerenchyma formation and waterlogging or hypoxia. Specific gravity measurement to estimate the changes of root porosity, and direct visual examination for the formation of aerenchyma in roots were two common methods used in previous studies. For dryland species, most studies focused on major crops such as corn, wheat, and pea, but the dynamics of aerenchyma development in turfgrass species are not well studied. The results of this study, examining the dynamics of root porosity and aerenchyma formation of two cool season grass species occur four weeks of waterlogging treatment, clearly showed the root porosity changes in relative to the formation of aerenchyma, and their relationship with waterlogging stress.

Aerenchyma portion and root porosity increased in both waterlogged TF and RB, showing both species have similar strategy of aerenchyma development in response to waterlogging. Because an aerenchyma system can provide benefits to the plant in terms of improving oxygen transport and reducing oxygen consuming cells, increased aerenchyma and root porosity can improve the waterlogging tolerance of these two species. Compared to the control plants, aerenchyma areas increased 48 and 31% in TF and RB at 4 WAT, respectively, while the root porosities increased 24 and 30% in TF and RB at 4 WAT, respectively. Because the formation of lysigenous aerenchyma may

involve programed cell death in the root cortex, the root functions such as water and mineral uptake and transport are influenced (Moog 1998). Aerenchyma formation involves a trade-off between maintaining root functions and reducing oxygen deficiency (Maricle and Lee 2002). The aerenchyma formation in RB appears to be more efficient in increasing root porosity than in TF, which could reduce the influence on physiological functions of waterlogged root.

Interestingly, during the four week long study, the aerenchyma increase was greater than the root porosity increase. From 1 WAT to 4 WAT, the aerenchyma area in waterlogged RB increased 138%, while the root porosity increased 50%. In waterlogged TF the aerenchyma area increased 55%, while the root porosity increased 40%. This finding indicates the formation of aerenchyma structures may not directly convert to the increase of root porosity. Furthermore, a root structure observation based study concluded that the aerenchyma formed during drained condition may not be sufficient to increase the oxygen supply during waterlogging in some species (Maricle and Lee 2002). Another important function of aerenchyma is to reduce the oxygen consumption and toxic metabolite production by reducing cells during waterlogging stress (Howes and Teal 1994, Drew 1997). Thus, the greater aerenchyma portion in waterlogged RB may reduce the oxygen requirement more compared with TF.

Previous studies indicated that lysigenous root aerenchyma does not typically form in well-drained dryland species such as maize, wheat, and barley, but may be induced by poor aeration (McDonald et al. 2001, Takahashi et al. 2014). Root aerenchyma formation was observed in drained TF and RB may indicate that TF and RB

have a similar but low oxygen stress threshold. The oxygen deficiency level could influence the formation of root aerenchyma, either in the formation rate or the aerenchyma area. Although the formation of aerenchyma in dryland species is less extensive than in wetland species (Armstrong 1979), the introduction of aerenchyma formation in dryland species usually occurs within several hours of perception of oxygen deficiency (Haque et al. 2010, Malik et al. 2003). Interestingly, the waterlogged RB did not produce a greater portion of root aerenchyma and porosity until 2 WAT. A possible explanation is that the RB has a lower oxygen requirement. Because the development of waterlogging induced oxygen deficiency is a slow process, it may not reach the low oxygen stress threshold of RB at 1 WAT. This feature may help to delay the aerenchyma formation during short-term waterlogging, and minimalize the loss of root function resulting by cell death during aerenchyma formation.

In conclusion, after four weeks of waterlogging treatment, both aerenchyma and root porosity increased in waterlogged RB and TF. The increase of aerenchyma portion in waterlogged RB was greater than in waterlogged TF, which may contribute to a better oxygen transportation in waterlogged RB and a lower oxygen consumption rate. Furthermore, the oxygen deficiency stress threshold was found lower in RB, which may contribute to a better waterlogging tolerance when waterlogging level is light.

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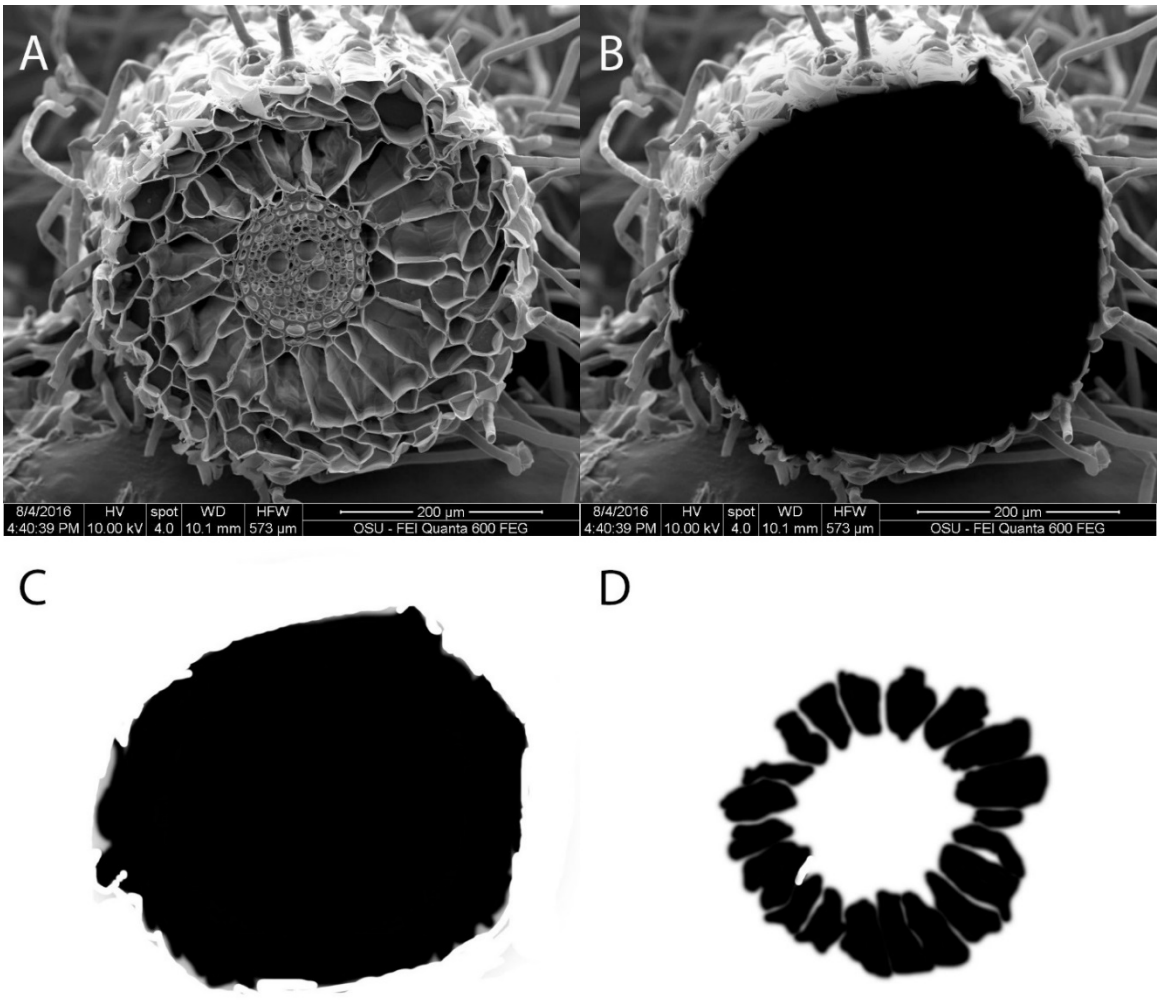

Fig. 4.1. Representative images used in digital quantification of aerenchyma area. (A) Original digital image of a tall fescues root cross section; (B) root cross section covered with black ink on a new image layer; (C) total cross sectional area; (D) aerenchyma spaces. Percentage of aerenchyma (44%) was calculated by dividing the number of pixels in (C) (pixels: 104932) by the number of pixels in (D) (pixels: 46917).

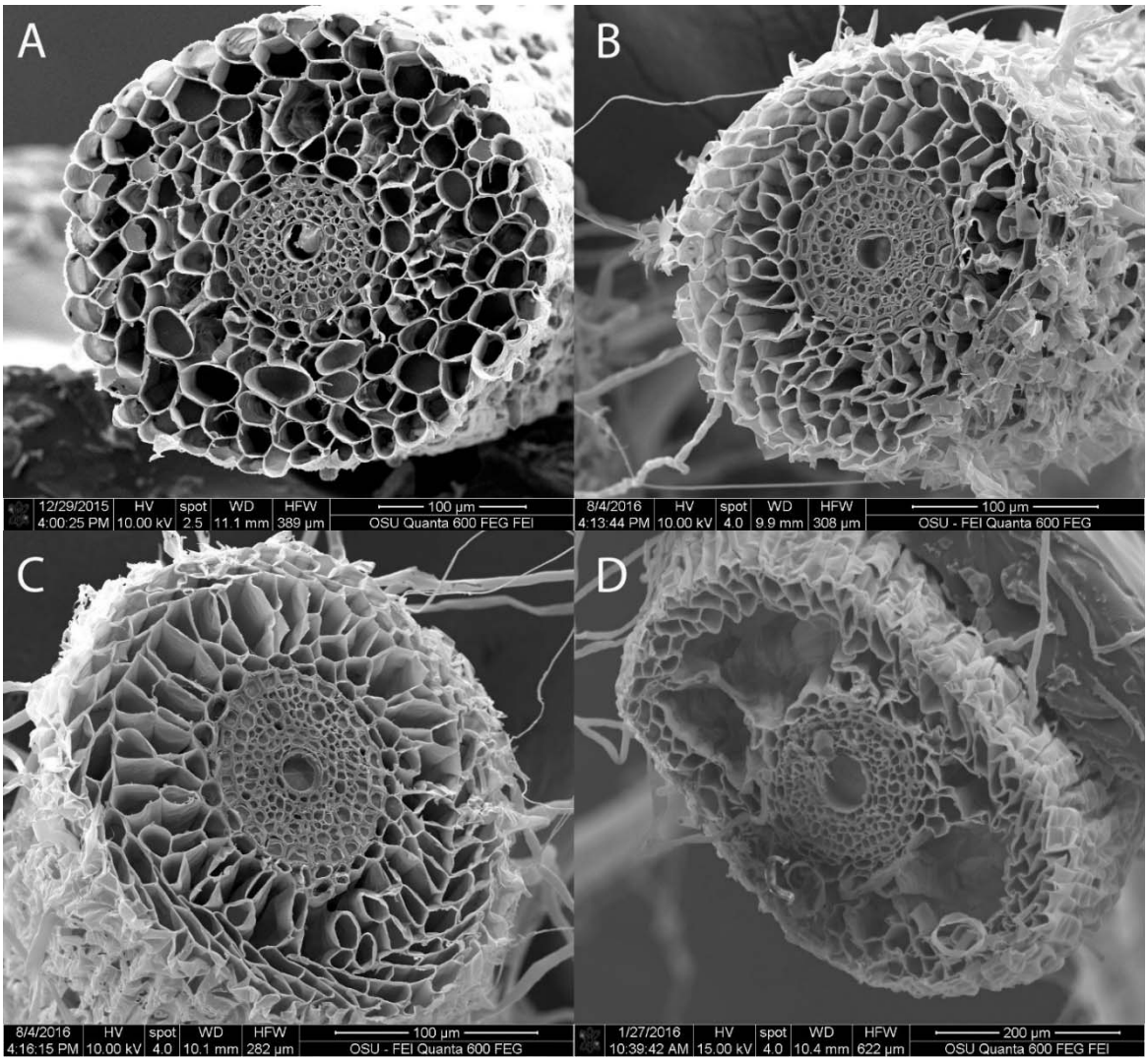

Fig. 4.2. Images of root cross sections in roughstalk bluegrass. Pictures are examples of root cross sections with 0 (A, control), 18 (B, control), 34 (C, waterlogging), and 51 % (D, waterlogging) aerenchyma by area. Aerenchyma occurred in both control and waterlogged plant roots.

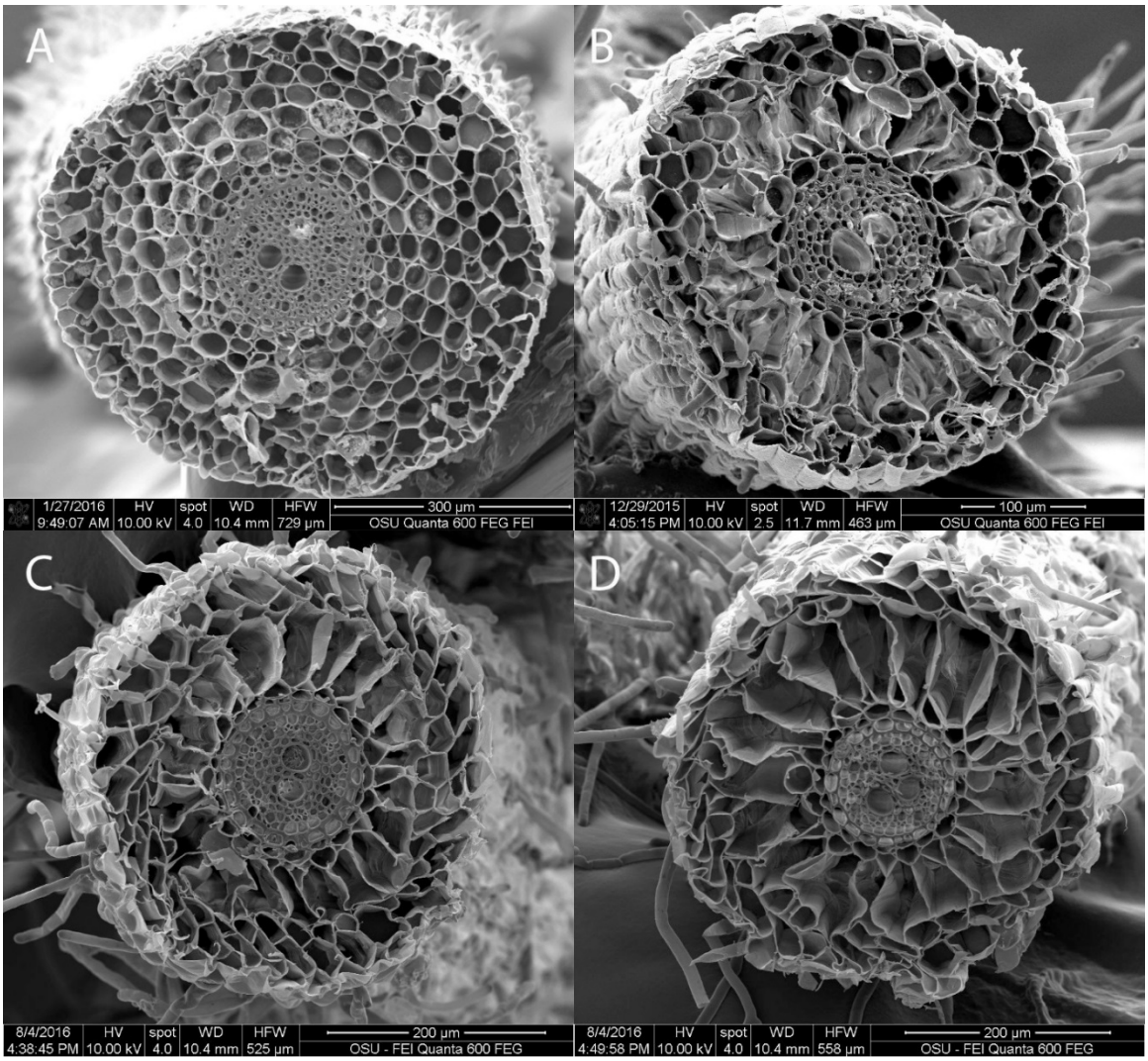

Fig. 4.3. Images of root cross sections in tall fescue. Pictures are examples of root cross sections with 0 (A, control), 24 (B, control), 29 (C, waterlogging), and 48 % (D, waterlogging) aerenchyma by area. Aerenchyma occured in both control and waterlogged plant roots.

Table 4.1. Soil redox potential reading during experiment.

Duration (Week)	Eh (mV)[a]	
	nor	wl
1	368	289
2	344	290
3	305	280
4	318	263

[a]nor = no treatment control, wl = waterlogging treatment

Table 4.2. Mean squares from the analyses of variances for changes in root porosity and aerenchyma area under different duration of waterlogging treatments.

Duration	Sources	df	Root porosity	Aerenchyma area
1 week	Species	1	13.78**	174.94***
	Treatment	1	34.03***	270.24***
	Species×Treatment	1	5.28*	70.50***
	Residuals	28	9.85*	223.30***
2 week	Species	1	22.78***	0.34
	Treatment	1	175.78***	362.73***
	Species×Treatment	1	1.53	2.98
	Residuals	28	14.91***	21.58***
3 week	Species	1	0.03	72.80***
	Treatment	1	247.53***	334.53***
	Species×Treatment	1	6.28*	4.92
	Residuals	28	17.81***	30.96***
4 week	Species	1	21.13***	331.64***
	Treatment	1	276.13***	860.84***
	Species×Treatment	1	3.13	5.29*
	Residuals	28	11.16**	38.14***

[a]P-value: *** < 0.001 < ** < 0.01 < * < 0.05

Table 4.3. Changes of percentage of aerenchyma in the root cross section by area during the 4 week greenhouse study. Data represent the mean of 8 replications.

Population	Treatment[a]	Aerenchyma area[b] (%)			
		Week 1	Week 2	Week 3	Week 4
TF	nor	14aA	23bA	24bA	23bA
	wl	22aB	28bB	31bcB	34cC
RB	nor	13aA	21bA	28cA	29cB
	wl	16aA	28bB	34cB	38cC

[a]: nor = no treatment control; wl = waterlogging treatment.
[b]: data were represented as the percentage of aerenchyma by area in root cross section.
[c]: Means followed by the same lower case letter in the row and upper case letter in the column are not significant based on Duncan's multiple range test at 0.05 probability.

Table 4.4. Changes of root porosities (%) during the 4 week greenhouse study. Data represent the mean of 8 replications.

Population	Treatment[a]	Root porosity (%)			
		Week 1	Week 2	Week 3	Week 4
TF	nor	19aA	23abAB	24bAB	25bA
	wl	22aA	28bC	28bB	31bB
RB	nor	19aA	21aA	22aA	23aA
	wl	20aA	26bBC	29bB	30bB

[a]: nor = no treatment control; wl = waterlogging treatment.
[b]: Means followed by the same lower case letter in the row and upper case letter in the column are not significant based on Duncan's multiple range test at 0.05 probability.

CHAPTER 5
GENERAL CONCLUSIONS

These studies were conducted to gain a better understanding of roughstalk bluegrass (*Poa trivialis* L.) (RB) invasion in Oregon grass seed production fields, which may contribute to better roughstalk bluegrass management in cool season grass seed production. Studies were conducted in a germination chamber and a greenhouse with simulated waterlogging or oxygen deficiency conditions. Comparisons of physiological responses to waterlogging were conducted between roughstalk and tall fescue (*Festuca arundinacea* Schreb.) (TF) in seed germination, early establishment, and mature stages. For the last stage, adaptive responses to waterlogging were exanimated in morphological, metabolic, and anatomical aspects. The results revealed a number of physiologically significant growth characteristics that can explain the better waterlogging tolerance of RB, and its invasion in waterlogged cool season grass seed production fields.

Although oxygen is required by seed germination of either TF or RB, different responses to waterlogging and temperature were observed between RB and TF. Compared to TF, the influence of waterlogging on seed germination was less in RB. Although the total germination were similar between RB and TF, RB seed germinated more quickly than TF under hypoxia conditions. It is likely that the germination of RB seed requires a lower oxygen concentration. This lower oxygen requirement may contribute to earlier RB germination during short term waterlogging.

The influence of waterlogging during the early establishment stage was greater for RB than for TF. Waterlogging reduced seedling biomass more in RB than in TF. The

reduced growth activity in RB also reduced the anaerobic metabolism. A benefit of reduced anaerobic metabolism during oxygen deficiency is to reduce cytoplasm acidosis and the following cell damage (Drew 1997).

Differences in several morphological adaptive characters were identified between waterlogged TF and RB. Root development in response to waterlogging treatment was the most distinguishable morphological trait between the two species. Root length increased by 6% in waterlogged TF, but decreased by 42% in waterlogged RB. The root dry biomass was reduced 43 and 13% in TF and RB, the root systems were thinner in TF than in RB. The vertical root distribution of RB is shallower than TF, which makes the oxygen absorption in RB greater than TF, because the soil oxygen concentration in the upper soil layer is greater than in deep soil layers. Waterlogging reduced leaf number and plant height more in TF than RB, but the reductions in turf quality and aboveground dry biomass were not different between these two species.

Increases in alcohol and lactic acid fermentation were identified in the root of waterlogged TF and RB, but not in the leaves. This finding indicated that the waterlogging induced oxygen deficiency may only influence the roots, but not the leaves. The increases of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) activities were less in roots of waterlogged RB, which indicated a lower fermentation rate compared with waterlogged TF. Lower fermentation activity produces less toxic metabolites, and causes less damage to the plant cells. Because accumulation of toxic metabolites in low-oxygen stressed cells is one of the major mechanisms of waterlogging

induced cell injury, lower fermentation activity during waterlogging may contribute to the better waterlogging tolerance of RB.

Water soluble carbohydrates (WSC) concentration was reduced in roots of waterlogged TF and RB, but not in the leaves. However, the reduction in neither waterlogged TF nor RB increased with the increase of waterlogging duration. WSC are important substrates for maintaining fermentation, and the root is one of the major WSC sinks in plant. Because the WSC concentration reached a stable level during the study, it may indicate a new balance between WSC production and consumption was reached in the waterlogged plant. Reductions in WSC concentration were not different between TF and RB, thus the WSC is not a limiting factor that influences the performances of waterlogged TF or RB.

The maximum photosynthesis rates were reduced by the waterlogging treatments in both TF and RB plants, but was not different between the species. The gas exchange characteristics including stomatal conductance and intercellular CO_2 concentration in waterlogged plants were not different across treatments or species. An important photosynthetic pigment, chlorophyll, decreased in waterlogged TF and RB at all times measured, which could be a reason for the reduction in maximum photosynthesis rates. Although waterlogging did not induce different responses in photosynthetic capacity between the two species, the treatment reduced leaf number and plant height more in RB than in TF. Because photosynthesis mainly takes place in leaves and may be influenced not only by leaf area but also by leaf age, leaf position and leaf structure (Araus et al. 1986, Koike 1988, Kitajima et al. 2002), these morphological characters in leaf

development may help to reduce the waterlogging influence on total photosynthetic production in RB.

Root aerenchyma formation was observed in both drained and waterlogged plants, which indicates waterlogging is not the only reason of aerenchyma formation in these two species. Aerenchyma formation and root porosity increased in waterlogged RB and TF, which are considered not only to increase the gas diffusion efficiency but also reduce the oxygen consumption. The aerenchyma portion in waterlogged RB was greater than in waterlogged TF, which may contribute to better oxygen transportation and lower oxygen consumption rate in waterlogged RB root.

In summary, responses in total seed germination, seedling emergence, turf quality, and aboveground biomass to waterlogging treatment were not different between TF and RB, which indicates that waterlogging induced oxygen deficiency may not be the only reason that contributes to the RB invasion in waterlogged fields. However, some metabolic, morphological, or anatomical adaptive characteristics were different between waterlogged TF and RB, which may contribute to a better waterlogging tolerance in RB. These characteristics include quick seed germination, lower oxygen requirement during establishment, thicker and shallower root system, lower fermentation under low oxygen stress, and larger aerenchyma areas. In terms of competition with TF or other cool season grass species, the less influenced germination of RB during waterlogging may result in a quick domination in waterlogged areas, because seed germination can affect many aspects of plant ecology including individual plant survival, population dynamics, and competitive interactions (Orrock and Christopher 2010). Furthermore, the lower oxygen

consumption and fermentation rate, and the better root gas diffusion may help RB survival waterlogging conditions for a longer term, and outcompete other species. For weed management purposes, reducing waterlogging areas in the field may reduce the advantage of RB in competition with other grass seed crops.

The results of this study improved our understanding of RB invasion in cool season grass seed production fields. Furthermore, the detailed study about the physiological responses in RB and TF will not only contribute to our understanding of the adaptations of grass species to low-oxygen stress but also provide potential knowledge to develop new waterlogging tolerant cool season grass cultivars. Further studies are required for a better understanding of RB invasion in the waterlogged fields. Firstly, the responses of RB to other waterlogging induced stresses such as salinity, soil nutrient leaching or oxidative stress (post-waterlogging) should be studied for a more accurate waterlogging effects evaluation. Secondly, the studies in RB should be extended to the reproduction stage. Studies of waterlogging influence on seed production in both weeds and crops can provide important information for estimating the influence of waterlogging on the seed bank and the seed yield.

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