8-13-2024

Salinity tolerance in the Elymus Genus

Hunter Pegram
Mississippi State University, hbpegram@gmail.com

Salinity tolerance in the *Elymus* Genus

By

Hunter Pegram

Approved by:

Jesse I. Morrison (Major Professor)
Brian S. Baldwin
Keri D. Jones
J. Brett Rushing
Michael S. Cox (Graduate Coordinator)
Scott T. Willard (Dean, College of Agriculture and Life Sciences)

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant and Soil Sciences (Agronomy)
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

August 2024

Name: Hunter Pegram

Date of Degree: August 8, 2024

Institution: Mississippi State University

Major Field: Plant and Soil Sciences

Major Professor: Jesse I. Morrison

Title of Study: Salinity tolerance in the *Elymus* Genus

Pages in Study 49

Candidate for Degree of Master of Science

ABSTRACT

Most crop species are highly sensitive to elevated levels of soil salinity. Increased soil salination has become one of the most detrimental environmental factors limiting agricultural productivity. Agricultural commodity losses due to salinity are currently estimated to be 12 billion USD per year and are expected to increase every year as more acreage is affected by salination. The *Elymus* genus is composed of approximately 150 species of grasses with geographic distribution that spans the globe. *Elymus* species are commonly used for revegetation, wildlife habitat, and erosion control. While increased tolerance to soil salinity has been reported that Argentine wheatgrass (*Elymus scabrifolius* (Doll) J.H. Hunz.)) and sand couchgrass (*Elymus farctus* L.) it is currently unknown if the ability to tolerate increased soil salinity exists in four popular North American *Elymus* species including southeastern wildrye [*Elymus glabriflorus* (Vasey ex L.H. Dewey)], Canada wildrye (*Elymus canadensis* L.), Virginia wildrye (*Elymus virginicus* L.), and riverbank wildrye (*Elymus riparius* Wiegand). In this study, tolerance to salinity was evaluated in these four species at the seed germination and seedling stage. The germination test was performed by watering seeds with 0, 100, 200, 300, and 400mmol NaCl and placing them in a

controlled environment chamber. The tolerance level was determined by germination rate. The seedling stage test was performed by irrigating seedlings with the same salinity levels in a greenhouse and monitoring injury and biomass yield reduction. This research will help identify salinity tolerance in these popular *Elymus* species at different life stages, allowing land managers and producers to make informed species selection decisions for revegetation, grazing, erosion control or habitat management when soil salinity is an issue.

DEDICATION

I would like to dedicate this thesis my wife, best friend, and biggest supporter, Alexandra Pegram, for all of the love and support. I would also like to dedicate this thesis to God Almighty, for giving me strength and wisdom throughout this process.

ACKNOWLEDGEMENTS

I would like to thank my parents, Tonya and Brad Pegram and my sister Kaitlyn Pegram for their support and love throughout this entire process. I would like to thank my major professor Dr. Jesse Morrison and my committee members Dr. Brian Baldwin, Dr. Keri Jones, and Dr. Brett Rushing for their help and guidance along the way. Lastly, I Would like to thank my fellow graduate students and student workers: Dylan Hollowell, Calyn Adams, Jared McLaurin, Eli Robb, Luke Noah, Jacob Shutt, and Grey Davis for there time and effort towards this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Soil salinity is a major global concern in the agriculture sector, as an estimated 1 billion hectares of land are affected worldwide, and the volume is increasing at a rate of 10% annually (Fageria et al., 2012). Salinity stress is an issue in nearly all irrigated parts of the world and can also be found in non-irrigated crops and rangelands (Carter, 1975). Salinity conditions are also negatively impacting 20% of all irrigated land, with some estimates being up to 50% (Pitman & Läuchli, 2002).

Increased salinity affects plant growth mainly via water stress, nutritional imbalance, salt stress, or a combination of all these factors. All of these can impair the plant's growth and development at physiological and biochemical levels. There have been small improvements in salinity tolerance over the last several decades through conservation selection and breeding techniques. The way that crops are selected for salinity tolerance is typically based on different agronomic characteristics, these include yield, survival, plant height, leaf area, leaf injury, relative growth rate, and relative growth reduction (Ashraf and Harris, 2004).

Tolerance to salinity will be vital for all commodity crop species as affected acres continue to increase. Similarly, tolerance in broad collections of perennial species like the *Elymus* genus will also be important given their importance to ecosystems and wildlife. Another factor influencing the importance of salinity tolerance in perennial grasses is the relative frequency that these species - both native and introduced - are planted into marginal, reclaimed,

or revegetated areas. Most species in the *Elymus* genus are not known for having salinity tolerance. It is important for endemic salinity tolerance to be identified and selected for in these species – specifically native ecotypes – so that native grasslands and ecosystems can be preserved or restored.

A defining characteristic of many species in the *Elymus* genus is the ability to establish quickly from seed, making them ideal for erosion control. Developing salinity tolerance in these species at both the seed germination and seedling growth stages will allow the continued use of multiple species in the various scenarios where they are desired.

CHAPTER II

OBJECTIVES

The increasing prevalence and severity of soil salination is a major concern in the agriculture and natural resources sector. Currently, soil salination affects approximately 1 billion hectares of arable land worldwide and is increasing every year. The goal of this research project was to identify any tolerance to salinity in four popular species from the *Elymus* genus at the seed germination and early seedling stages, thus increasing their utility for land managers and potentially leading to the development of highly salinity-tolerant germplasm lines. These objectives were achieved by screening seed of Virginia, Canada, riverbank and southeastern wildrye for germination under four levels of salinity (100, 200, 300, 400 mmol). Seedlings grown from the same seedlots were also evaluated for tolerance to the same salinity conditions at the three to four-leaf growth stage.

CHAPTER III

LITERATURE REVIEW

North American Native Plant Species

Both warm and cool-season native plant species play an important part in conservation and agricultural production. Native grasses are popularly used in long-term plantings as protective or filter areas to reduce soil erosion, improve water quality, and provide habitat for native wildlife. The most popular warm- and cool-season native grasses are often deep-rooted, long-lived perennials that can tolerate low pH, low fertility, and drought (ESG NRCS, 2011). Cool-season grasses have an advantage over warm-season grasses for erosion control because they establish dense stands in just a year or two (Lynn, 2004). Riveroat (*Chasmanthium latifolium* (Michx.) Yates) is a popular cool-season perennial grass. It is often found in bottomlands and can be used for good forage and hay (ROFG, 2024). Fowl mannagrass (*Glyceria striata* (Lam.) Hitchc.) is another cool-season rabidly establishing native grass, it has high palatability for horses and cattle (Darris, 2006). A popular *Elymus* species are Siberian wildrye (*Elymus sibiricus* L). It is a cool season perennial that is grown for pasture and hay in northern China (Zhao et al., 2017). Argentine wheatgrass (*Elymus scabrifolius*) is an important forage crop in Argentina. It has also shown the ability to be used as forage in areas with salinity stress (Jauregui et al., 2017).

Elymus

Elymus is the largest genus of Triticeae tribe, which includes several important cereal, forage, and range species (Barkworth, 2021). Species of this genus are among the most important cool-season forage species, and they can be used as a potential gene source for wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) breeding (Dewey, 1984; Barkworth and Dewey, 1985), including for drought and salt tolerance (Colmer et al., 2006; Nevo & Chen, 2010).

Adaptations of plants to salinity is critical at both the germination and seedling stage. Germination of sand-couchgrass (*Elymus farctus* (Viv.) Runemark ex Melderis) seed under the influence of nutrients and salinity was evaluated at salinity concentrations of 0, 50, 100, 200, and 300 mmol NaCl in water or on a full nutrient solution containing 6 mmol N present in the form of ammonium, nitrate, or ammonium nitrate. The results showed a significant decrease in germination at treatment levels beyond 100 mmol, with a critical threshold of 55 mmol NaCl before negative impacts on germination were detectable. In this study the addition of nutrients in the form of ammonium, nitrate, and ammonium nitrate significantly affected salinity response, in some cases making the inhibitory effect of salinity non-significant (El-Katony, Khedr, and Soliman, 2015).

Canada Wildrye

Canada wildrye (*Elymus canadensis* L.) is a highly desirable grazed forage for livestock and can also be harvested and preserved as hay. It is also used in seed mixtures to prevent erosion where revegetation is needed. This especially applies to places where construction work has been done. It can also be used in flower arrangement because it produces flowers from summer through winter (Bush, 2002). Canada wildrye is used for feed, ornamental purposes, wildlife, and livestock. It is used ornamentally as a good bunchgrass. Wildlife use it for nesting

material, and the seeds can feed small mammals and birds. It is also very palatable and nutritious for livestock and serves as a source for early spring grazing for cattle. Ideal growing conditions are in part shade, and moist, well-drained soil. It can tolerate sandy, loam, clay or limestone soil conditions and is drought tolerant. It is usually found in grasslands, ravines, depressions, open woodlands, ditches, and fencerows. It is found in 43 of the 50 states and in some parts of Canada (Lloyd-Reilley, 2010).

Canada wildrye is a short-lived cool-season grass found growing on shaded stream banks and can also be found on sand shores and dunes. Canada wildrye has adapted moderate salt tolerance. It can be used as a cool season pasture crop and a cool season grass in a native seed mix. When being planted, they should be about .63 to 1.27 centimeters deep into the ground. The seeding rate per acre is 11.2085 kilograms of pure live seed per hectare. The purity of this seed is usually around 95% with an 85% germination rate. When establishing Canada wildrye, it should not be grazed for the first year (Lloyd-Reilley, 2010).

Riverbank Wildrye

Riverbank wildrye (*Elymus riparius* Wiegand) is a native, perennial, cool-season grass that grows from 0.9 to 1.5 meters tall. It has one singular spike that ranges between 10 and 15 centimeters in length and has 2 additional smaller spikelets. Riverbank wildrye grows best in moist soils and in full to partly shade areas. Its fruiting season runs from July to September (MWFER, 2024). Riverbank wildrye is a grass or a grass-like plant that is in the Poaceae family that grows best in moist and shady soils. It grows well in organic, clay, loam, and sandy soils and is found in 28 states in the United States and in parts of Canada (NPONA, 2015).

Southeastern Wildrye

Southeastern wildrye (*Elymus glabriflorus* (Vasey ex L.H. Dewey) Scribn & C.R. Ball) is a relatively short-lived cool-season perennial grass native to North America. Seed produced by southeastern wildrye is often fast to germinate with a high germination rate. These characteristics make it a good option for revegetation and erosion prevention. Southeastern wildrye is important for land managers because it is used in developing plans that combine the benefits of wildlife habitat with production agriculture. (Belt et al., 2013). Southeastern wildrye is an important revegetation species that can help prevent soil erosion in highly erodible areas. Until recently, southeastern wildrye was described as a subspecies of Canada wildrye, and later as a subspecies of Virginia wildrye before being categorized as a distinct species (Rushing et al., 2016). Southeastern wildrye is a cool-season perennial commonly found along rights-of-way, woodland boundaries, and fallow fields across the Southeast, Midwest, and East Coast (Rushing & Baldwin, 2015). Southeastern wildrye is an adaptable species, tolerant of full or part sun, wet or dry, acidic or neutral, and coarse to fine-textured soils. It has high crude protein, low neutral detergent fiber, and low acid detergent fiber (Rushing et al., 2016).

Southeastern wildrye is an important native grass for restoration and soil stability. Seed characteristically germinates quickly and has a high germination rate (70%) making it useful in highly erodible areas where rapid establishment is necessary. (Belt et al., 2013). Southeastern wildrye is also preferred as a resource to protect water quality because it requires little to no fertilization to establish and maintain. Southeastern wildrye complements warm-season plants by providing soil coverage when warm-season grasses are dormant, and the seed remains highly viable for up to 10 years. Southeastern wildrye also can function similarly to other wildryes. For example, a mixture of Canada, Virginia, and riverbank wildrye with fringed bromegrass (*Bromus*

ciliatus L.) and fowl bluegrass (*Poa palustris* L.) provides cover for erosion control on up to a 2% grade (Belt et al., 2013).

Virginia Wildrye

Virginia wildrye (*Elymus virginicus* L.) is a palatable alternative as forage or hay for livestock. Virginia wildrye is suitable for effective erosion control and is an easy-to-grow native groundcover. The flowers can also be attractive in flower arrangements (Shadow, 2009) Virginia wildrye is a cool-season, perennial grass popularly used by birds and small mammals for nesting and denning material. It grows best in partly shady, moist soil and organic, clay, loam, and sandy soils. It is usually found along shady banks, fence rows, and open woodlands. Its geographical distribution ranges from Arizona to the east coast and ranges as far north as Quebec. (Shadow and Jensen, 2020). Virginia wildrye is a fair grazing crop for livestock and is often grazed from fall to early spring before reproduction is initiated. Virginia wildrye can also be a nutritious food source for large game such as deer, and when found in wetlands the seed is utilized by ducks and geese (Shadow and Jensen., 2020).

Virginia wildrye is also an effective cool-season component for seed mixtures with warm-season grasses for restoration and conservation plantings (Shadow, 2009). Virginia wildrye provides an important cool-season component to seed mixtures with warm-season grasses for significant ground coverage year-round on conservation and restoration plantings. The seed does not require any treatment and usually has an 85% germination rate. Virginia wildrye should be planted in the spring or fall at 0.63 to 1.27 centimeters in heavy soils and 2.54 centimeters depth in sandy soils. There should not be any fertilizer put on Virginia wildrye in its first year of establishment, but after the first year, it may need to be fertilized depending on soil conditions (Shadow and Jensen, 2020).

Salinity

Soil salinity develops as a result of many different factors, including poor water management, high evaporation, heavy irrigation, and exposure to seawater. Most plants are very susceptible to salinity, these plants are called glycophytes. Plants that are tolerant to salt are called halophytes. Halophytes can grow at salinities over 250 mmol NaCl in contrast to glycophytes which usually cannot grow in salinity over 100 mmol NaCl (Tuteja, 2015). Most crops are highly sensitive to salinity in the soil, making soil salination one of the most severe environmental factors currently limiting agricultural productivity. The negative monetary impact of salinity on agriculture is estimated to be 12 billion USD per year and is expected to increase as soils are further affected.

There have been two characteristic responses to the negative impacts of soil salination: engineering the environment by irrigation and drainage management to reduce or mitigate salinity or increasing plant salinity tolerance (Pitman & Läuchli, 2002). Among the major effects of soil salinity on commodity crops, the most quantifiable are reductions in feed, fiber, and forage quality, decreased seedling establishment, decreased grain yield, and reduced seed germination. This is a major issue considering 95 million hectares in the world are currently impacted by salinity conditions. It is believed that the best way to overcome salinity is to evaluate wild species to find tolerant genotypes (Masoudi, et al., 2010). High salinity when there is a drought makes the effects of the drought more considerable because salinity can affect germination and seedling growth by preventing water uptake or by toxic effects of sodium and chloride ions in the germinating seed. (Akbarimoghaddam et al., 2011). Sodium also displaces potassium which is an essential plant nutrient.

Salinity levels in agricultural land are increasing every year, and the urgency to develop plants that are salt tolerant is increasing as well. The best strategy for producing a plant that is successfully salt tolerant is by breeding. The results of plant breeding for salinity tolerance have increased yield significantly in salinity soils (Wani et al., 2020).

There have been many improvements in selecting for salt-tolerant crops; however, the main reason for the limited success in finding salt-tolerant crops is that genetic diversity within many of these species are very low. Another reason there is not a lot of movement in salinity stress is that breeders typically focus on improving yield and quality rather than improving stress tolerances such as salt tolerance (Ashraf & Akram, 2009). Now that the world is facing salinity issues in much of its farmland, plant breeders will start looking to improve salt tolerance and stress tolerance in many crops.

Salinity Tolerance in the Triticeae Tribe

The current literature is lacking for examples of salinity tolerance in the *Elymus* genus and the Triticeae tribe as a whole. Chen et al. (2023) evaluated 50 wild Siberian wildrye (*Elymus sibiricus* L.) accessions collected from Russia and Asia for tolerance to salinity after plants have developed 5-8 tillers. Plants were submerged in a 200 mmol NaCl Hoagland solution for 14 days. Results identified three salt-tolerant accessions providing germplasm for breeding salt-tolerant cultivars (Chen et al., 2023).

Argentine wheatgrass is an important forage in salinity environments. Some tolerant genotypes show growth reduction in salinity stress, but continued to develop new leaves after showing symptoms where non-tolerant plants did not (Zabala et al., 2020). Some other Triticeae plants that have displayed salt tolerance at 200 mmol NaCl are Duhurian wildrye (*Elymus*

dachurius Turcz. Ex Grieseb), mammoth wildrye (*Leymus* racemosus (Lam.) Tzvelev.), and Altai wildrye (*Leymus angustus* (Trin.) Pilg.) (Gorham et al., 1994).

Conclusion

While salinity tolerance characteristics vary considerably within the Triticeae tribe, the genetic resources for improvement of key crops – including wheat and barley – are of great importance to the agriculture sector. There is very little information in the literature regarding salinity tolerance in the *Elymus* genus, especially among the species native to North America. Establishing the current level of salinity tolerance among *Elymus* species is an important first step in identifying potential gene sources for interspecific breeding including opportunities for intraspecific improvement breeding to increase salinity tolerance. Increased salinity tolerance will broaden the utility of these North American native species in grassland, forage, and conservation settings.

CHAPTER IV

EXPERIMENT I: SALINITY TOLERANCE IN ELYMUS SP. AT THE SEED GERMINATION STAGE

Introduction

Salinity is a major problem in many plants due to the stress it can put on them. Most plants are most venerable to salinity when they are seeds or when they are small seedlings. If the plant does not have any tolerance towards the salt in the soil and water, then it will not be able to grow into a mature plant and will often die.

This research was conducted in controlled environment laboratory on the main campus of Mississippi State University, Starkville, MS. All seedlots were obtained new from the identified sources prior to the first experiment and were used for each consecutive experiment. When not in use, seed were stored in a commercial freezer at ambient conditions of -18°C.

Materials and Methods

This experiment was conducted from 15 Feb- 8 Mar 2023 and was repeated from 15 June- 12 July 2023. Four species of the *Elymus* genus were screened for germination at five salinity levels (0, 100, 200, 300, 400 mmol NaCl). Seedlots of Virginia wildrye, Canada wildrye, and riverbank wildrye were obtained from Roundstone Native Seed LLC (Upton, KY), and southeastern wildrye ‘Copiah’ foundation seed was collected from research plots at Mississippi State University H.H. Leveck Animal Research Center near Starkville, MS. Bulk seedlots were processed in a drum-style debearder (Q-Sage, Mt. Pleasant, MI) to remove awns and then

homogenized using an impeller-type forced air fractionating aspirator to separate full, dense seed from chaff and empty or partially filled seed. Six replicate subsamples of 50 seed from each seedlot were placed on two thicknesses of filter paper in 110 mm petri dishes.

Salinity treatments were prepared by dissolving ultra-pure sodium chloride into deionized water. Salinity treatments (5 ml) were applied to each dish and were the only moisture necessary to complete the study. Replicate dishes of each seedlot were stacked together and maintained in a controlled environment chamber set to 12h daylength, 25°C day/15° night (AOSA recommended; AOSA 2014). Germination was recorded every other day for 21 days. Individual seed were considered germinated after emergence of the root radicle (≥2 mm) and coleoptile (≥3 mm). Germinated seed were removed from dishes after being recorded. Seed remaining after 21 days were counted to establish total germination percentage.

Germination data was analyzed using PROC GLIMMIX (SAS Institute, Cary, NC) following methodology described by Sileshi (2012) and Gianinetti (2020). Main effects were considered significant at $\alpha = 0.05$.

Results

Statistical analysis of germination showed a significant effect due to experiment ($p = 0.0227$) due to this, germination data will be presented separately by experiment. While germination was observed at the 300 mmol level for southeastern wildrye only, there was a lack of germination at the 400 mmol level across all species. Due to this, no data are presented for the 400 mmol treatment.

Experiment 1 Germination

Canada wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control, 100 mmol and 200 mmol treatments producing germination percentages of 26.7, 4.3, and 0.3, respectively (LSD= 5.8, Figure 4.1).

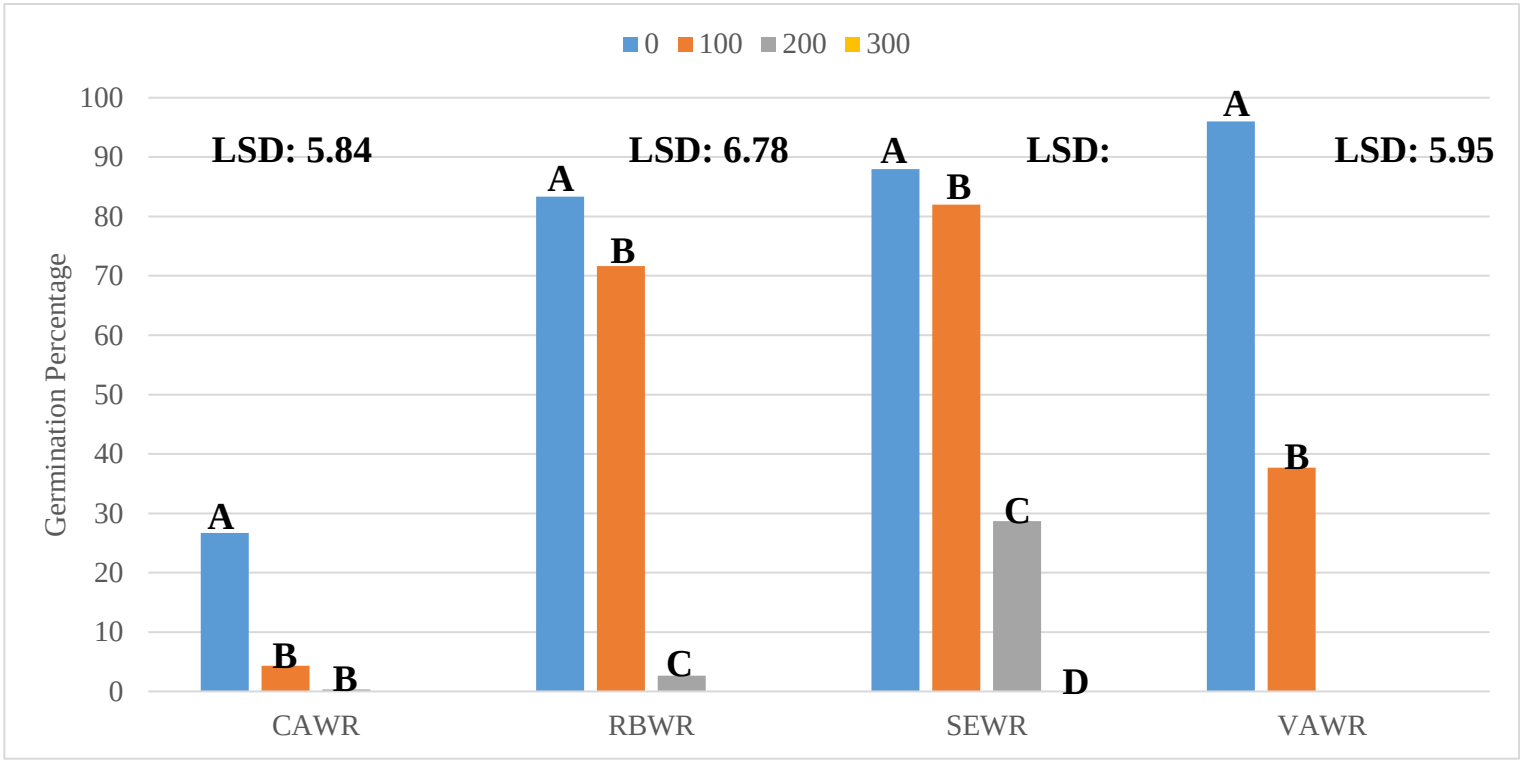

Figure 4.1 Germination percentage in experiment 1

Germination percentage in experiment 1 of four Elymus species at 0, 100, 200, and 300 mmol NaCl in 110 mm petri dishes in a controlled environment chamber (12-hour daylength, 25°C day/15° night) (CAWR= Canada wildrye, RBWR= riverbank wildrye, SEWR= southeastern wildrye, VAWR= Virginia wildrye).

Riverbank wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control, 100 mmol and 200 mmol treatments producing germination percentages of 83.3, 71.7 and 2.7, respectively (LSD= 6.7, Figure 4.1).

Southeastern wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control, 100 mmol, 200 mmol and 300 mmol treatments producing germination percentages of 88.0, 82.0, 28.7, and 0.3, respectively (LSD= 5.6, Figure 4.1).

Virginia wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control and 100 mmol treatments producing germination percentages of 96.0 and 37.7, respectively (LSD= 5.9, Figure 4.1).

Experiment 2 Germination

Canada wildrye

There was a significant effect on seed germination due to salinity treatment (P<.0001). Generally, germination percentage decreased as salinity level increased from untreated control to 200 mmol. Germination percentage decreased significantly from 100 mmol to 200 mmol and remained unchanged from 200 mmol to 300 mmol. Overall, the untreated control, 100 mmol,

200 mmol and 30 0mmol treatments producing germination percentages of 28.0, 5.0, 0.7 and 1.0, respectively (LSD= 5.5, Figure 4.2).

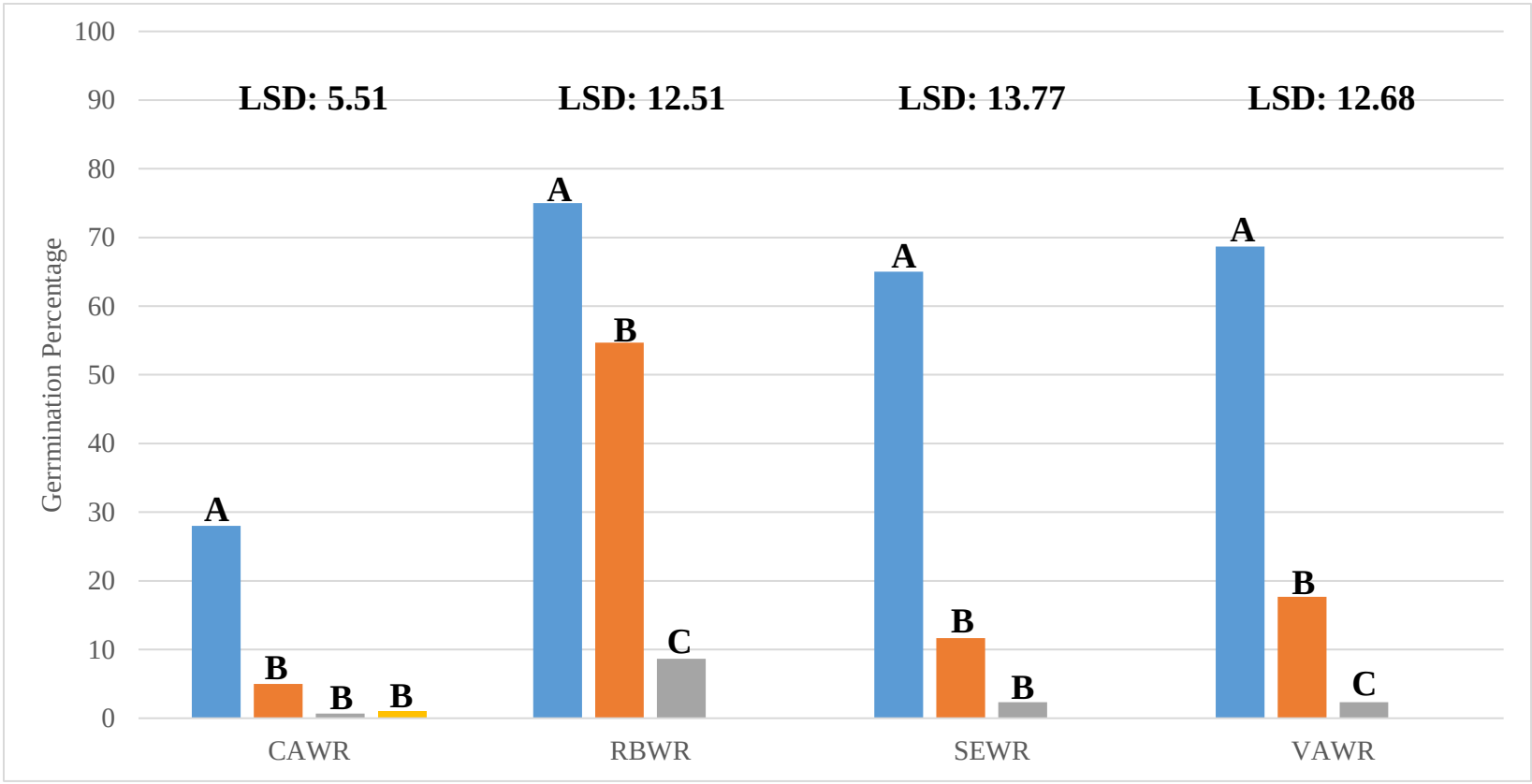

Figure 4.2 Germination percentage in experiment 2

Germination percentage in experiment 2 of four *Elymus* species at 0, 100, 200, and 300 mmol NaCl in 110 mm petri dishes in a controlled environment chamber (12-hour daylength, 25°C day/15° night) (CAWR= Canada wildrye, RBWR= riverbank wildrye, SEWR= southeastern wildrye, VAWR= Virginia wildrye).

Riverbank wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control, 100 mmol and 200 mmol treatments producing germination percentages of 75.0, 54.7, and 8.7, respectively (LSD= 12.5, Figure 4.2).

Southeastern wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Generally, germination percentage decreased significantly as salinity level increased. There was a significant decrease in germination at 100mmol salinity and germination remained unchanged at 200 mmol. Overall, the untreated control, 100mmol and 200mmol treatments producing germination percentages of 65.0, 11.7, and 2.3, respectively (LSD= 13.7, Figure 4.2).

Virginia wildrye

There was significant effect on seed germination due to salinity treatment (P<.0001). Germination percentage decreased significantly as salinity level increased, with the untreated control, 100 mmol and 200 mmol treatments producing germination percentages of 68.7, 17.7, and 2.3, respectively (LSD= 12.6, Figure 4.2)

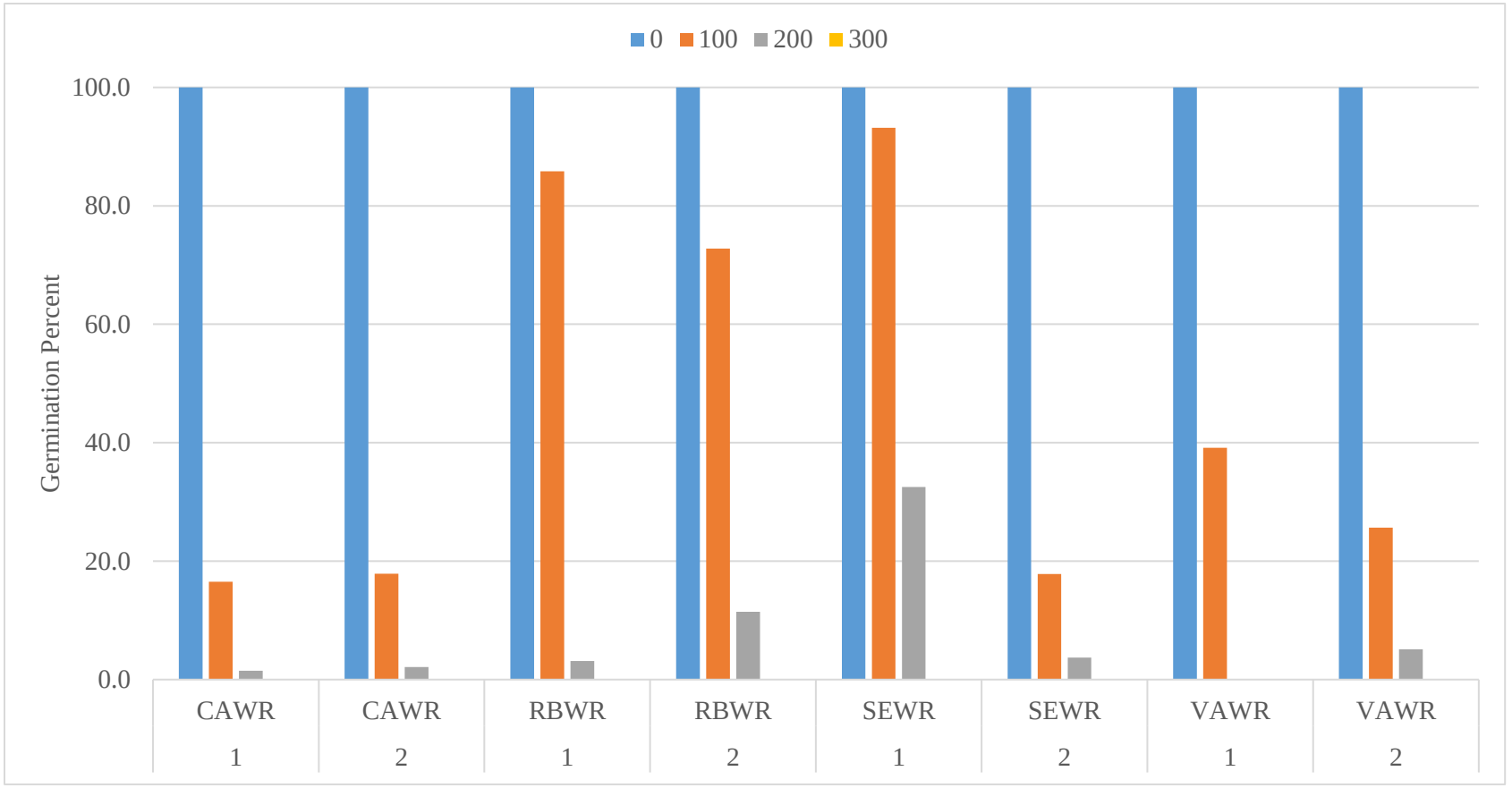

Figure 4.3 Germination rates as a percentage of untreated control.

Germination rates as a percentage of untreated control. (CAWR 1= Canada wildrye experiment 1, CAWR 2= Canada wildrye experiment 2, RBWR 1= riverbank wildrye experiment 1, RBWR 2= riverbank wildrye experiment 2, SEWR 1= southeastern wildrye experiment 1, SEWR 2= southeastern wildrye experiment 2, VAWR 1= Virginia wildrye experiment 1, VAWR 2= Virginia wildrye experiment 2)

Discussion

Overall, all species showed some level of salt tolerance at the seed germination stage. Each of the species was able to germinate at the 100 mmol salinity level. Generally, for each species, germination percentage significantly decreased from the 100 mmol to 200 mmol treatment. This supports the findings in (El-Katony, Khedr, and Soliman, 2015) where the authors reported a significant decrease in germination at treatment levels beyond 100 mmol. Southeastern wildrye showed an increased tolerance to elevated salinity, as it was able to germinate at 100 and 200 mmol both years while also being able to germinate at 300 mmol in the first year.

Germination decreased significantly form year 1 to year 2 regardless of treatment level. Statistical analysis of germination showed a significant effect due to year ($p = .0227$). This could be because the same seedlots were used for both years, and likely displayed the effects of extended after-ripening in year 2. Seedlots were stored in a commercial freezer at an ambient temperature of -18°C in between study replications to limit this effect. Seedlots were carried over in order to limit the risk of receiving a new seedlot that did not perform as well or displayed different salinity tolerance. By using the same seedlots, we were able to maintain continuity between experiments, allowing us to confirm that response to treatment was not due to variation in seedlot genetics.

CHAPTER V

EXPERIMENT II: SALINITY TOLERANCE IN *ELYMUS* SP. AT THE SEEDLING STAGE

Introduction

Soil salinity is a major inhibitor of many crop and range species, generally due to a lack of tolerance to saline conditions. Commonly, plants are most venerable to salinity seed germination and early seedling stages. If the plant does not have any tolerance towards the salt in the soil and water, then it will not be able to grow into a mature plant and will often die.

This research was conducted in a controlled environment greenhouse located on at Mississippi State University R.R. Foil Plant Science Research Center, Starkville, MS. All seedlots were obtained new from the identified sources prior to the first experiment and were used for each consecutive experiment. When not in use, seed were stored in a commercial freezer at ambient conditions of -18°C.

Materials and Methods

This experiment was conducted from 31 Jan – 20 Feb 2023, and was repeated from 5 Feb – 26 Feb 2024. Four species of the *Elymus* genus were screened at the seedling stage for tolerance to five salinity levels (0, 100, 200, 300, 400 mmol NaCl). Seedlots of Virginia wildrye, Canada wildrye, and riverbank wildrye were obtained from Roundstone Native Seed LLC (Upton, KY), and southeastern wildrye seed was collected from research plots at Mississippi State University H.H. Leveck Animal Research Center near Starkville, MS. Bulk seedlots were processed in a drum-style debearder (Q-Sage, Mt. Pleasant, MI) to remove awns and then

homogenized using an impeller-type forced air fractionating aspirator to separate full, dense seed from chaff and empty or partially filled seed.

Fifty-cell plug trays (Sure Roots 50, T.O. Plastics) were filled with peat-based potting media (Sunshine #4, Sungro Horticulture) and the first twenty cells of each end were seeded with three seeds of a single Elymus species. Each twenty-cell section was an experimental unit, and each species/treatment combination was represented with four replications. Each plug tray was placed into a standard, nonperforated propagation tray. All irrigation, fertilization, and treatment applications were made by filling the propagation tray. The seedlings were watered as needed and then fertilized with a liquid solution of 200ppm N using a balanced fertilizer (Peter's 20-20-20) 14 days after being planted. The seedlings were thinned another 12 days later leaving a single plant per cell. Greenhouse was maintained at ambient daylength, and temperature fluctuated between 20°-30°C daytime and 12°-24°C nighttime.

Salinity treatments were prepared by dissolving ultra-pure sodium chloride into deionized water. When ≥90% of seedlings had reached the three-leaf stage, salinity treatments (4L) were applied to each tray and allowed to stand for four hours before being poured out. Trays were arranged in a random order within the greenhouse and were irrigated as needed for the duration of the experiment. Total mortality was recorded 21 days after treatment application. Mortality was assessed by visual evaluation and scored as a 1 (alive) or 0 (dead) for each sampling unit (seedling). Following evaluation, plant biomass was removed by cutting plants flush with the top of the plug tray. Harvested biomass was weighed, dried in a forced air oven at 55°C for seven days, and reweighed to assess moisture content and dry matter yield. Ground biomass samples were evaluated for mineral content by Waypoint Analytical Laboratory (Memphis, TN). Biomass

accumulation, mortality, and mineral content data were analyzed using PROC GLIMMIX (SAS Institute, Cary, NC).

Results

Statistical analysis of biomass accumulation showed a significant effect due to year ($p<.0001$) due to this, biomass accumulation data are presented separately by year.

Year 1 Biomass Accumulation

Canada wildrye

There was significant effect on biomass accumulation due to salinity treatment ($P<.0001$). Biomass accumulation increased significantly as salinity level increased from untreated control to 100 mmol. Biomass accumulation decreased significantly from 100 mmol to 200 mmol and from 200 mmol to 300 mmol but remained unchanged from 300 mmol to 400 mmol. The untreated control, 100 mmol, 200 mmol, 300 mmol, and 400 mmol had biomass yields of 240.03, 193.60, 177.50, 144.73, and 141.25 mg, respectively (LSD= 29.35, Figure 5.1).

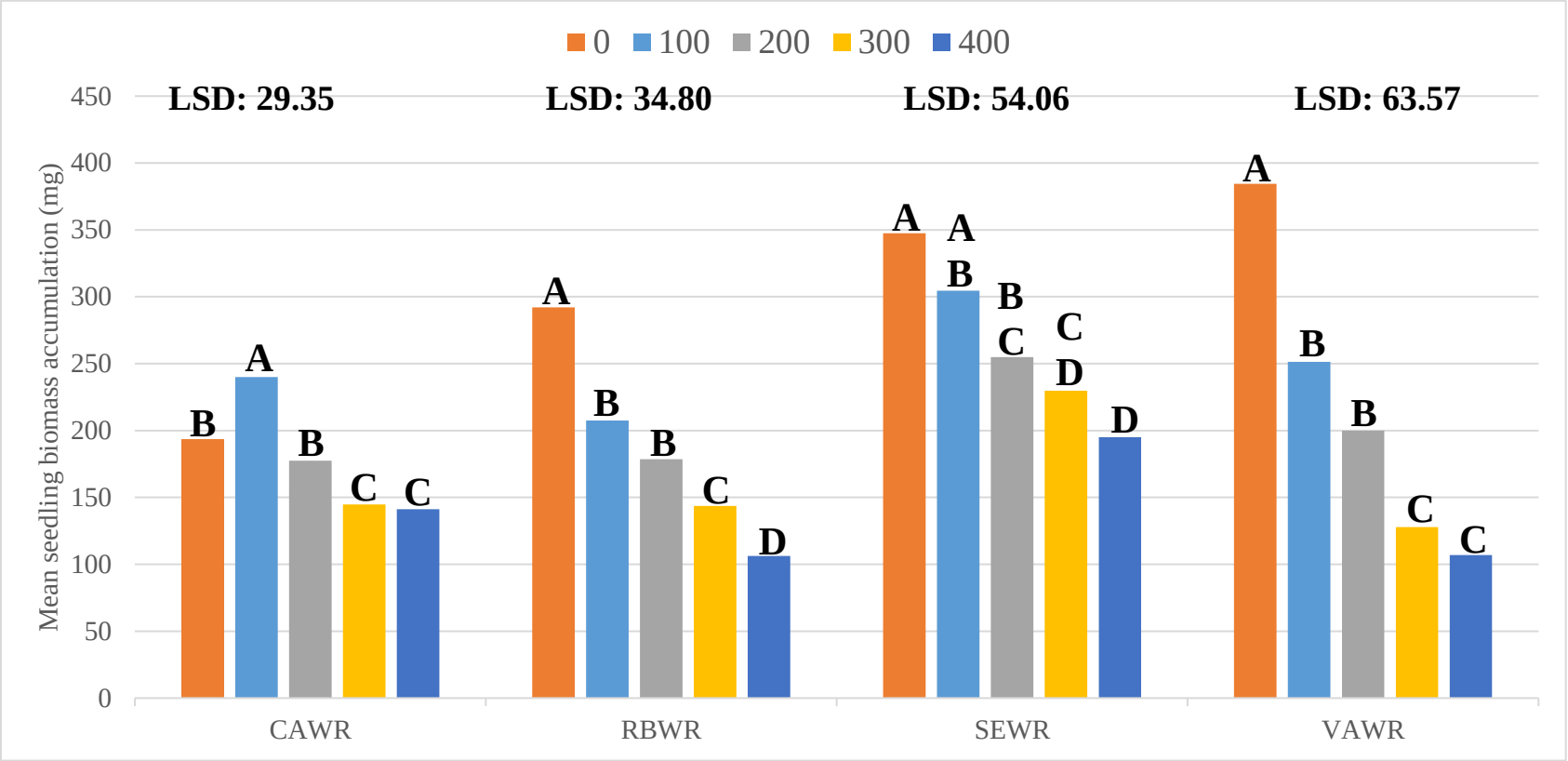

Figure 5.1 Mean seedling biomass accumulation (mg) in year 1

Mean seedling biomass accumulation (mg) in year 1 of four *Elymus* species 21d after treatment with different levels of salinity-impacted irrigation water in a greenhouse that was maintained at ambient daylength, and temperature fluctuated between 20°-30°C daytime and 12°-24°C nighttime (CAWR= Canada wildrye, RBWR= riverbank wildrye, SEWR= southeastern wildrye, VAWR= Virginia wildrye).

Riverbank Wildrye

There was significant effect on biomass accumulation due to salinity treatment ($p<.0001$). Biomass accumulation decreased significantly as salinity level increased, with the untreated control, 100, 200, 300, and 400 mmol treatments producing biomass accumulations of 292.13, 207.50, 178.65, 143.53, and 106.18 mg, respectively (LSD= 34.80, Figure 5.1). There was no difference in biomass accumulation between 100 and 200 mmol treatments as well as no difference between survivors of the 300 and 400 mmol treatments.

Southeastern wildrye

There was significant effect on biomass accumulation due to salinity treatment ($p=.0002$). there was a significant decrease in biomass accumulation at treatment levels of 200 mmol and greater when compared to the untreated control. There was no significant difference in biomass accumulation between the untreated control and 100 mmol treatments. Generally, biomass accumulation decreased as salinity level increased, with the untreated control, 100, 200, 300, and 400 mmol treatments producing biomass accumulations of 347.50, 304.63, 255.00, 229.80, and 195.00 mg, respectively (LSD= 54.06, Figure 5.1).

Virginia wildrye

There was significant effect on biomass accumulation due to salinity treatment ($p<.0001$). Biomass accumulation decreased significantly as salinity level increased, with the untreated control, 100, 200, 300, and 400 mmol treatments producing biomass accumulations of 384.48, 251.33, 200.00, 127.90, and 106.93 mg, respectively (LSD= 63.57, Figure 5.1). There was no difference in mean accumulation between 100 and 200 mmol treatments as well as no difference between 300 and 400 mmol treatments.

Year 2 Biomass Accumulation

Canada wildrye

There was no significant effect on biomass accumulation due to salinity treatment (p=0.6293). Biomass accumulation was numerically greatest at 100 mmol and numerically lowest at 400 mmol. Overall, biomass yield decreased slightly as salinity level increased. Biomass accumulation of untreated control, 100, 200, 300, and 400 mmol treatments were 100.28, 107.50, 107.05, 92.10, and 68.75 mg, respectively (LSD= 59.44, Figure 5.2).

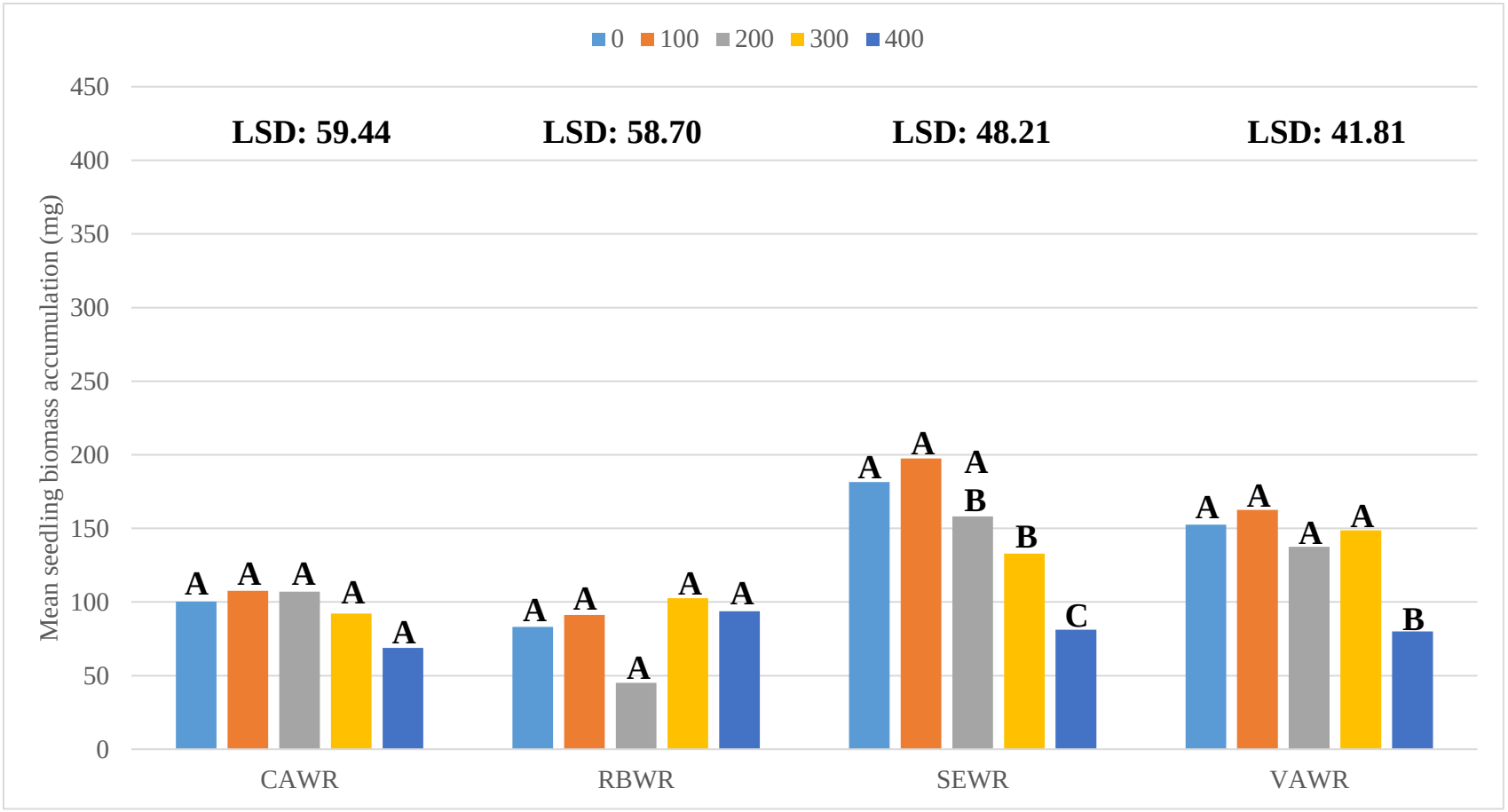

Figure 5.2 Mean seedling biomass accumulation (mg) in year 2

Mean seedling biomass accumulation (mg) in year 2 four *Elymus* species 21d after treatment with different levels of salinity-impacted irrigation water in a greenhouse that was maintained at ambient daylength, and temperature fluctuated between 20°-30°C daytime and 12°-24°C nighttime (CAWR= Canada wildrye, RBWR= riverbank wildrye, SEWR= southeastern wildrye, VAWR= Virginia wildrye).

Riverbank wildrye

There was no significant effect on biomass accumulation due to salinity treatment (p= .3064). Biomass accumulation was numerically greatest at 300 mmol. There was a numerical decrease in biomass accumulation at 200 compared to all other treatments (LSD= 58.70, Figure 5.2).

Southeastern wildrye

There was significant effect on biomass accumulation due to salinity treatment (p= .0011). Biomass accumulation significantly decreased at 400 mmol compared to untreated control, 100, and 200 mmol treatments (LSD= 48.21, Figure 5.2).

Virginia wildrye

There was significant effect on Biomass accumulation due to salinity treatment (p= .006). Biomass accumulation decreased significantly at 400 mmol compared to all other treatments (LSD= 41.81, Figure 5.2).

Mortality

There was no significant effect on seedling mortality due to year (p=.4722), so data were pooled across years. There was significant effect on mortality due to variety (p=.0032) as well as significant difference across salinity rate (p<.0001). Overall, as treatment level increased plant mortality significantly increased as well.

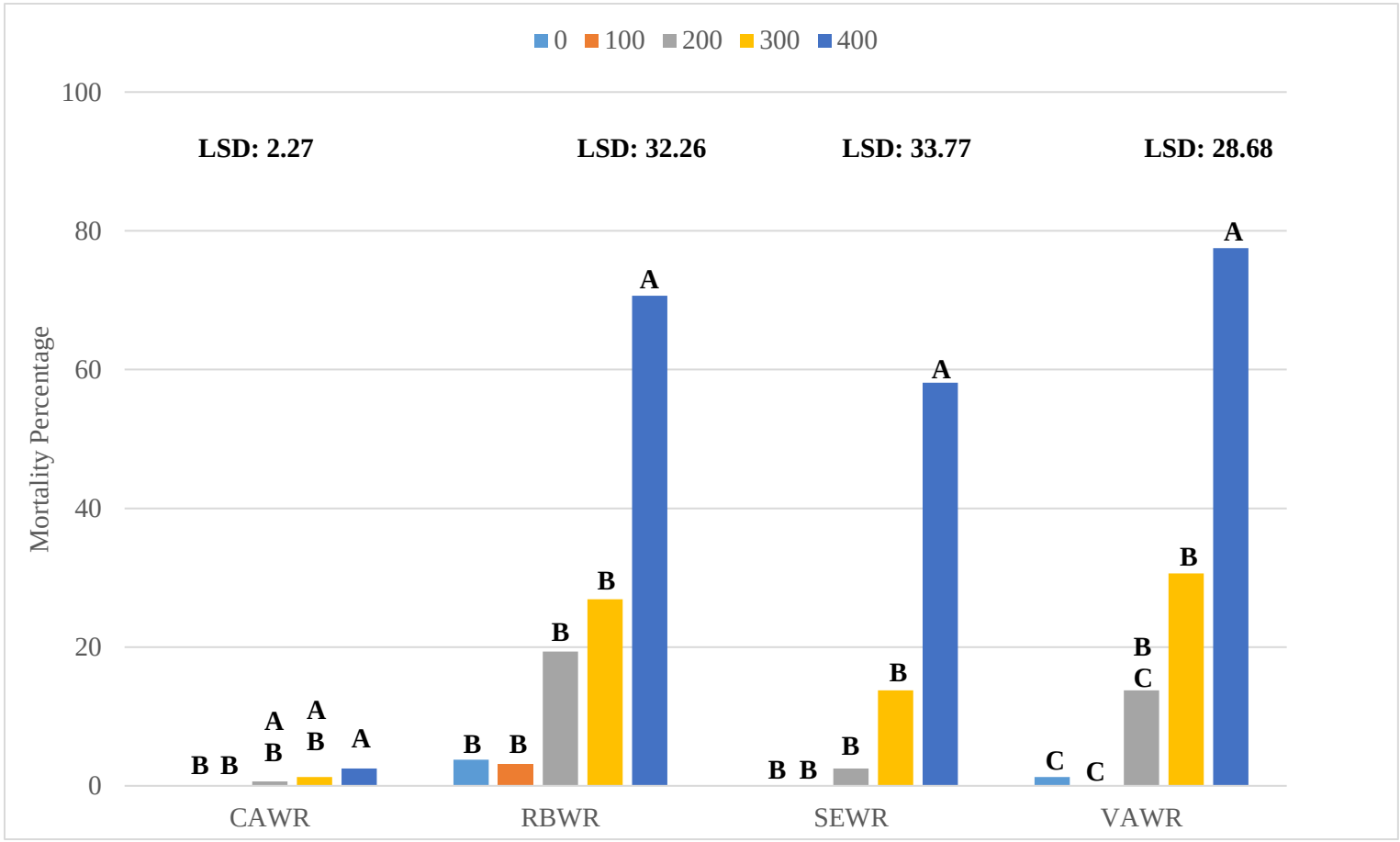

Figure 5.3 Mortality percentage pooled across years

Mortality percentage pooled across years in four *Elymus* species 21d after treatment with different levels of salinity-impacted irrigation water in a greenhouse that was maintained at ambient daylength, and temperature fluctuated between 20°-30°C daytime and 12°-24°C nighttime (CAWR= Canada wildrye, RBWR= riverbank wildrye, SEWR= southeastern wildrye, VAWR= Virginia wildrye).

Canada wildrye

There was no significant effect on mortality due to treatment rate (p=.1444). Overall, as treatment levels increased mortality increased, mean mortality percentage at untreated control, 100, 200, 300, and 400 were 0, 0, .63, 1.3, and 2.5%, respectively (LSD=2.27, Figure 5.3).

Riverbank wildrye

There was a significant difference in mortality in Riverbank wildrye due to treatment rate (p=.014). Overall, as treatment levels increased mortality increased. Mean mortality percentage at untreated control, 100, 200, 300, and 400 were 3.75, 3.13, 19.38, 26.88, and 70.63%, respectively (LSD=32.26, Figure 5.3).

Southeastern wildrye

There was significant difference in mortality in Southeastern wildrye due to treatment rate (p=.026). Overall, as treatment levels increased mortality increased. Mean Mortality percentage at untreated control, 100, 200, 300, and 400 were 0, 0, 2.5, 13.75, and 58.13%, respectively (LSD=33.77, Figure 5.3).

Virginia wildrye

There was significant difference in mortality in Virginia wildrye due to treatment rate (p=.004). Overall, as treatment levels increased mortality increased. Mean mortality percentage at untreated control, 100, 200, 300, and 400 were 1.25, 0, 13.75, 30.63, and 77.50%, respectively (LSD=28.68 Figure 5.3).

Mineral content of biomass

Year, species, and treatment were considered main effects in the analysis of plant mineral content. There was a significant year x variety interaction for multiple mineral components, however there was no interaction for sodium (Na) or chloride (Cl) content in harvested biomass (Table 5.2, 5.3, 5.4).

Table 5.2 Analysis of variance for sodium (Na)

Source	Df	SS	MS	F	Pr>F
Model	11	335.07	30.46	36.12	<.0001
Error	148	124.82	0.84		
Year	1	54.95	54.95	65.16	<.0001
Variety	3	32.51	10.83	12.85	<.0001
Treatment	4	246.65	61.66	73.11	<.0001
Year*Variety	3	0.94	0.31	0.38	<.7709
Corrected Total	159	459.89			

Content of harvested biomass from seedling salinity tolerance study.

Table 5.3 Analysis of variance for chloride (Cl)

Source	Df	SS	MS	F	Pr>F
Model	11	396.53	36.04	90.19	<.0001
Error	148	59.15	0.39		
Year	1	52.60	52.60	131.60	<.0001
Variety	3	9.53	3.17	7.95	<.0001
Treatment	4	332.27	83.06	207.82	<.0001
Year*Variety	3	2.11	0.90	1.76	<.1566
Corrected Total	159	455.68			

Content of harvested biomass from seedling salinity tolerance study.

Table 5.4 Analysis of variance of harvested biomass mineral content from seedlings of four species† of Elymus 21 days after treatment with 0, 100, 200, 300 and 400 mM NaCl impacted irrigation water.

Source	N	S	P	K	Mg	Ca	Na	Cl	B	Zn	Mn	Fe	Cu	Al
	----------%----------								----------ppm----------					
Year	***	***	***	NS	***	***	***	***	***	***	***	NS	***	NS
Species	***	***	***	***	NS	NS	***	***	*	***	***	NS	***	*
Treatment	***	***	NS	***	***	***	***	***	***	***	***	*	*	NS
Species*Treatment	***	***	***	***	*	NS	NS	NS	NS	***	*	*	***	NS

†Elymus species included Canada, Riverbank Southeastern, and Virginia wildrye
NS, Not significant
* Significant at the .05 probability level.
*** Significant at the .001 probability level.

Sodium and chloride accumulation in harvested biomass followed a generally linear trend, as mineral concentrations irrigation water increased, tissue concentration also increased (Figure 5.4, 5.5, 5.6, 5.7). While sodium uptake did not appear to reach a plateau in any species x treatment combination, it is worth noting that the species x treatment combinations with the greatest sodium concentration in harvested biomass also reported increased levels of plant mortality.

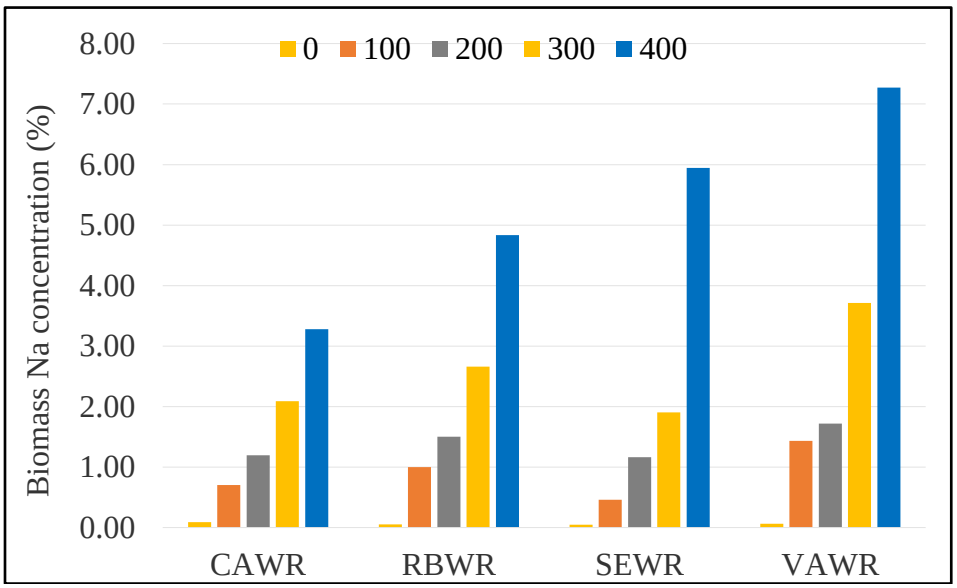

Figure 5.4 Year 1 sodium content of biomass

Year 1 sodium content of biomass in four *Elymus* species harvested 21d after treatment with different levels of salinity-impacted irrigation water. CAWR= Canada wildrye (LSD=0.91), RBWR= riverbank wildrye (LSD=0.45), SEWR= southeastern wildrye (LSD=1.09), VAWR= Virginia wildrye (LSD=0.85).

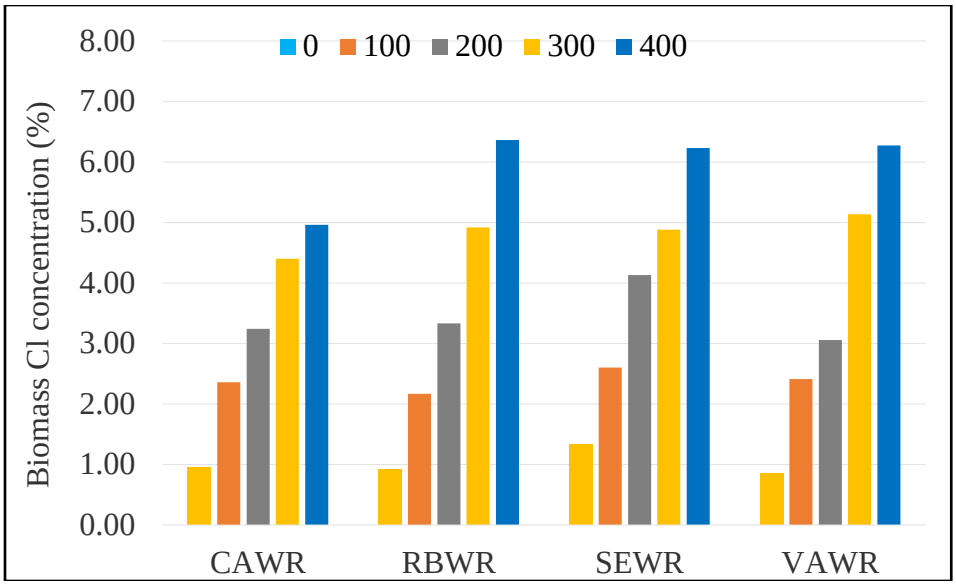

Figure 5.5 Year 1 chloride content of biomass

Year 1 chloride content of biomass in four *Elymus* species harvested 21d after treatment with different levels of salinity-impacted irrigation water. CAWR= Canada wildrye (LSD=0.90), RBWR= riverbank wildrye (LSD=0.48), SEWR= southeastern wildrye (LSD=0.52), VAWR= Virginia wildrye (LSD=0.80)

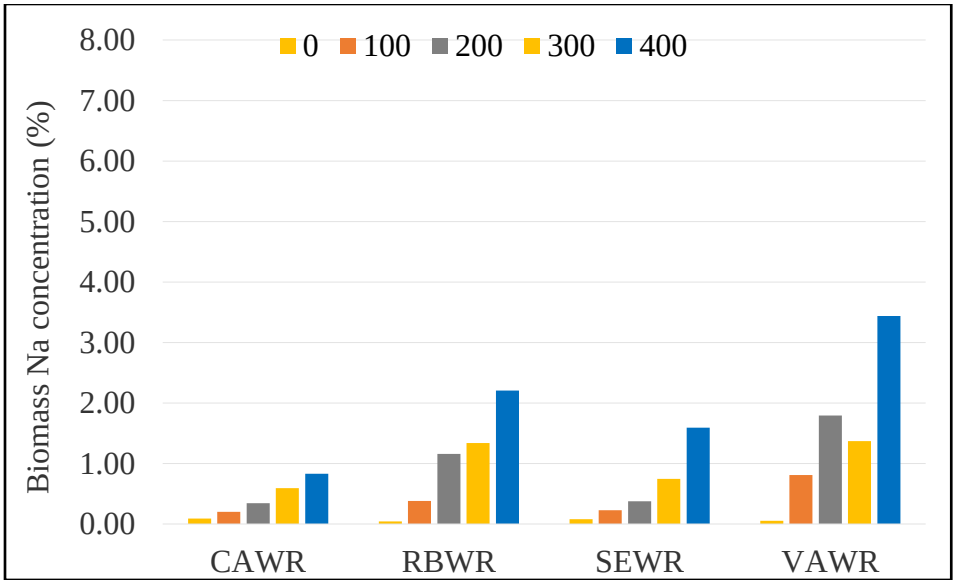

Figure 5.6 Year 2 sodium content of biomass

Year 2 sodium content of biomass in four *Elymus* species harvested 21d after treatment with different levels of salinity-impacted irrigation water. CAWR= Canada wildrye (LSD=0.25),

RBWR= riverbank wildrye (LSD=1.1), SEWR= southeastern wildrye (LSD=0.40), VAWR= Virginia wildrye (LSD=0.66).

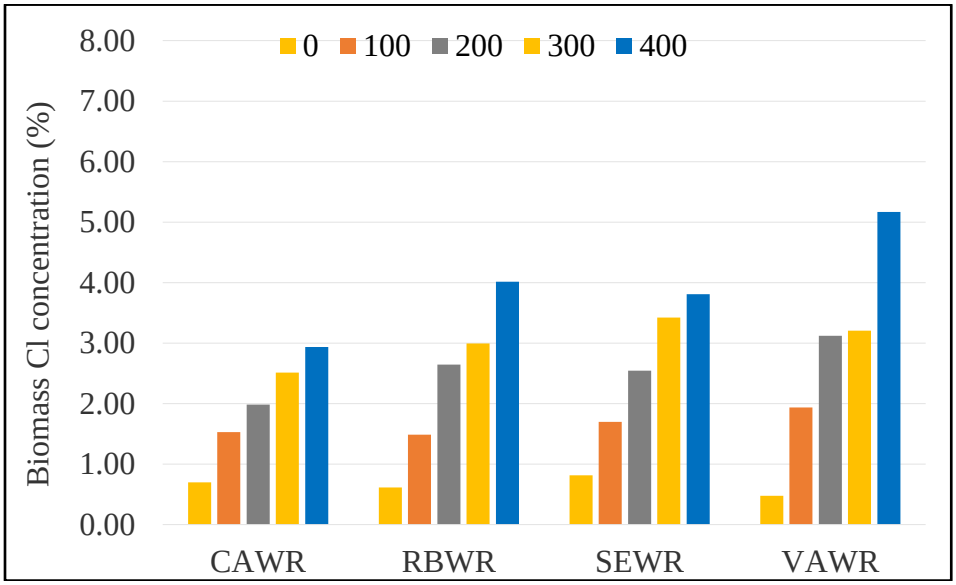

Figure 5.7 Year 2 chloride content of biomass

Year 2 chloride content of biomass in four *Elymus* species harvested 21d after treatment with different levels of salinity-impacted irrigation water. CAWR= Canada wildrye (LSD=0.56), RBWR= riverbank wildrye (LSD=1.07), SEWR= southeastern wildrye (LSD=0.66), VAWR= Virginia wildrye (LSD=0.76).

Discussion

While mortality was significant at increased treatment levels, all four of these *Elymus* species showed the ability to survive at up to 400 mmol salinity at the seedling stage. Generally, as treatment levels increased, biomass accumulation decreased in year 1, however, the results in year 2 were less distinct. In general, VAWR and SEWR produced greater yields across all treatments than CAWR and RBWR with few exceptions. In year 1, VAWR also showed a more severe reduction in biomass production as treatment levels increased.

Overall, biomass accumulation decreased from year 1 to year 2. Statistical analysis of biomass accumulation showed a significant effect due to year ($p<.0001$). This could be because

the same seedlots were used for both years, and likely displayed the effects of extended after-ripening in year 2. Seedlots were stored in a commercial freezer at an ambient temperature of -18°C in between study replications to limit this effect. Seedlots were caried over in order to limit the risk of receiving a new seedlot that did not perform as well or displayed different salinity tolerance. By using the same seedlots, we were able to maintain continuity between experiments, allowing us to confirm that response to treatment was not due to variation in seedlot genetics. A malfunctioning control system in the greenhouse caused mean ambient temperatures to be slightly higher (~4°C) in year 2, but this difference is not likely to cause the observed decrease in biomass accumulation. The commercial potting media used for seedling analysis was also different in year 2, but again, is unlikely to be the reason for decreased biomass accumulation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Canada, riverbank, southeastern, and Virginia wildrye were not previously reported to have any tolerance to saline conditions at the seed germination or seedling stages. All four species showed some ability to germinate and survive in saline conditions. The characteristics of rapid seed germination and high germination rates combined with salinity tolerance makes these species desirable for revegetation and erosion control. This shows that *Elymus* can be an option for revegetation in areas that deal with salinity problems in the soil and irrigation water. It also shows the advantage that these species could have for erosion control along streambanks, riverbanks, marshlands, and other potentially saline areas that are prone to erosion. Each of the four *Elymus* species were able to germinate in up to 200 mmol NaCl and were able to survive in saline conditions up to 400 mmol NaCl.

At the seed germination stage, riverbank and southeastern wildrye were able to consistently germinate at the 100 mmol treatment and they were also the best when it comes to germinating at the 200 mmol treatment. Canada wildrye also showed germination up to 200 mmol NaCl, although it had a decreased overall germination. Virginia wildrye germinated best at the untreated control but germination fell off quickly as treatment levels increased. Germination was also recorded at 200 mmol, but very few seed germinated at this treatment level and then only in the second experiment.

In the biomass accumulation experiment, southeastern wildrye and Virginia wildrye generally showed the highest biomass accumulation across all treatments. As the treatments increased biomass accumulation decreased across all varieties.

The low overall germination in Canada wildrye was expected, as the seedlot information provided on the tag reported a true germination percentage of 21%, with 78% hard seed (Appendix A, Seeedlot Information).

In the biomass mineral content analysis, there was a significant year*variety interaction, which is most likely attributable to issues with temperature regulation in the greenhouse, coupled with the use of a different potting media between years. All data for biomass mineral content can be found in Appendix A, Table A.1. As treatment levels increased the sodium and chloride found in the plants also increased.

This research is a start to developing highly saline-tolerant germplasm in the *Elymus* genus for revegetation purposes. Future research is necessary to explore the limits of salinity tolerance these species can achieve with further selection. There is need for other *Elymus* species to be evaluated for salinity tolerance as information is the literature is generally lacking.

REFERENCES

AOSA (Association of Official Seed Analysts). 2014. Rules for testing seeds. Available online at: htttp://www.aosaseed.com/aosa_rules_for_testing_seeds Verified August 1, 2016.

Ashraf, M. P. J. C., & Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Science, 166(1), 3-16.

Ashraf, M., & Akram, N. A. (2009). Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison. Biotechnology Advances, 27(6), 744-752.

Barkworth, M.E. 2021. Flora of North America. FNAA. Utah State University. Vol. 24

Barkworth, M.E. and D.R. Dewey. 1985. Genomically based genera in the perennial Triticeae of North America: Identification and membership. Amer. J. Bot. 72:767-776

Belt S, Rushing B, Tangren S. 2013. Plant guide for southeastern wildrye (Elymus glabriflorus). Beltsville (MD): USDA Natural Resources Conservation Service, Norman A. Berg National Plant Materials Center.

Bush, T. (2002) Plant fact sheet for Canada Wildrye (*Elymus canadensis*). USDA NRCS Rose Lake Plant Materials Center.

Carter, D. L. (1975). Problems of salinity in agriculture. In: Plants in saline environments (pp. 25-35). Springer, Berlin, Heidelberg.

Chen S, Feng J, Xiong Y, Xiong Y, Liu Y, Zhao J, Dong Z, Ma X, Yan L. Evaluation and Screening of Wild *Elymus sibiricus* L. Germplasm Resources under Salt Stress. *Agronomy*. 2023; 13(11):2675. https://doi.org/10.3390/agronomy13112675

Colmer, T.D., T.J. Flowers, and R. Munns. 2006. Use of wild relatives to improve salt tolerance in wheat. J. of Exp. Bot. 57 (5): 1059–1078. https://doi.org/10.1093/jxb/erj124

Darris, D. (2006) Tall Mannagrass (*Glyceria elata*) Plant Fact Sheet. USDA NRCS Plant Materials Center, Corvallis, Oregon.

Dewey, D.R. 1984. The genomic system of classification as a guide to intergeneric hybridization in the perennial Triticeae. Pp. 209-279 in J.R Gustafson (ed.). Gene Manipulation in Plant Improvement. Plenum Press, New York, NY, USA

El-Katony, T. M., Khedr, A. H. A. F., & Soliman, N. G. (2015). Nutrients alleviate the deleterious effect of salinity on germination and early seedling growth of the psammophytic grass *Elymus farctus*. Botany, 93(9), 559-571.

Elymus riparius (Riverbank Wild Rye) (2024). Retrieved from https://www.minnesotawildflowers.info/grass-sedge-rush/riverbank-wild-rye.

Establishing Native grasses. (n.d.). Retrieved from https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_017880.pdf

Fageria, N.K., Stone, L.F., Santos, A.B.d. (2012). Breeding for Salinity Tolerance. In: Fritsche-Neto, R., Borém, A. (eds) Plant Breeding for Abiotic Stress Tolerance. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30553-5_7

Gorham J. 1994. Salt tolerance in the Triticeae: K/Na discrimination in some perennial wheatgrasses and their amphiploids with wheat. Journal of Experimental Botany 45, 441 —447.

Gianinetti, A. (2020). Basic features of the analysis of germination data with generalized linear mixed models. Data, 5(1), 6.

Jauregui, C. G., Ruiz, M. D. L. Á., & Ernst, R. D. (2017). Tolerancia a la salinidad en plántulas de agropiro criollo (*Elymus scabrifolius*) y agropiro alargado (*Thinopyron ponticum*). Pastos y forrajes, 40(1), 29-36.

Lloyd-Reilley, J. (2010). Plant Guide: Canadian wildrye (Elymus canadensis L.) USDA Natural Resource Conservation Service

Lynn, A. (2004). Conservation Practice Fact Sheet. Comparing Warm-season and Cool-season Grasses for Erosion Control, Water Quality, and Wildlife Habitat. NRCS Field Service Center

Missouri Department of Conservation. Canada Wild Rye (n.d). Retrieved from https://mdc.mo.gov/discover-nature/field-guide/canada-wild-rye

Nevo, E., and G. Chen. 2010. Drought and salt tolerances in wild relatives for wheat and barley improvement. Plant, cell & environment, 33(4), 670–685. https://doi.org/10.1111/j.1365-3040.2009.02107.x

Pitman, M. G., & Läuchli, A. (2002). Global impact of salinity and agricultural ecosystems. In Salinity: Environment-plants-molecules (pp. 3-20). Springer, Dordrecht.

Purya, M., Ali, G., & Mehdi, A. (2010). Improving emergence and early seedling growth of two cool season grasses affected by seed priming under saline conditions. African Journal of Agricultural Research, 5(11), 1288-1296.

River Oats Field Guide (2024) retrieved from: (https://mdc.mo.gov/discover-nature/field-guide/river-oats).

Rushing, J. B., & Baldwin, B. S. (2015). Optimal sowing depth for southeastern wildrye (Elymus glabriflorus). Native Plants Journal, 16(3), 243-248.

Rushing, J. B., Saha, U. K., Lemus, R., Sonon, L., & Baldwin, B. S. (2016). Analysis of some important forage quality attributes of Southeastern Wildrye (*Elymus glabriflorus*) using near-infrared reflectance spectroscopy. American Journal of Analytical Chemistry, 7(09), 642.

Shadow, A., USDA NRCS East Texas Plant Material Center (2009) Plant Guide: Virginia wildrye (*Elymus virginicus* L.) USDA Natural Resource Conservation Center

Shadow, R.A and N.K. Jensen. 2020. Plant Guide for Virginia wildrye (Elymus virginicus L.). USDA-Natural Resources Conservation Service. Plant Materials Program.

Sileshi, G. W. (2012). A critique of current trends in the statistical analysis of seed germination and viability data. Seed Science Research, 22(3), 145-159.

Tuteja, N. (2007). Mechanisms of high salinity tolerance in plants. Methods in enzymology, 428, 419-438.

Virginia Wild Rye (n.d.). Retrieved from https://mdc.mo.gov/discover-nature/field-guide/virginia-wild-rye

Wani, S. H., Kumar, V., Khare, T., Guddimalli, R., Parveda, M., Solymosi, K., ... & Kavi Kishor, P. B. (2020). Engineering salinity tolerance in plants: Progress and prospects. Planta, 251(4), 1-29.

Zabala, J. M., Marinoni, L. D. R., Taleisnik, E. L., Ribero, G., & Schrauf, G. E. (2020). Salt tolerance in Argentine wheatgrass is related to shoot sodium exclusion. *Crop Science*, *60*(5), 2437-2451.

Zhao, X., Xie, W., Zhang, J., Zhang, Z., & Wang, Y. (2017). Histological characteristics, cell wall hydrolytic enzymes activity and candidate genes expression associated with seed shattering of *Elymus sibiricus* accessions. *Frontiers in Plant Science*, *8*, 606.

APPENDIX A

SEEDLOT SOURCE, PURITY, AND GERMINATION INFORMATION ANALYSIS OF VARIANCE FOR MINERAL NUTRIENT CONTENT OF BIOMASS

Seedlot Information

Virginia wildrye

Ordered from	Roundstone Native Seed, LLC. Upland, KY
Origin	Iowa
Test date	12/21
Pure seed	99.09
Total germ	99.00
PLS	98.10
Inert matter	0.91
Weed seed	0.00
Crop seed	0.00
Hard/Dorm	85.00
Germ	14.00

Seedlot label from roundsone Native Seed LLC

Canada wildrye

Ordered from	Roundstone Native Seed, LLC. Upland, KY
Origin	West Virginia
Test date	02/21
Pure seed	96.07
Total germ	99.00
PLS	95.11
Inert matter	3.93
Weed seed	0.00
Crop seed	0.00
Hard/Dorm	78.00
Germ	21.00

Seedlot label from roundsone Native Seed LLC

Riverbank wildrye

Ordered from	Roundstone Native Seed, LLC. Upland, KY
Origin	Pennsylvania
Test date	06/22
Pure seed	99.61
Total germ	92.00
PLS	91.64
Inert matter	0.39
Weed seed	0.00
Crop seed	0.00
Hard/Dorm	10.00
Germ	82.00

Seedlot label from roundsone Native Seed LLC

Southeastern wildrye

Collected by	Dr. Brian Baldwin and Dr. Brett Rushing
Origin	Starkville, MS

Seedlot information

Table A.1 Analysis of variance of harvested biomass mineral content from seedlings of four species† of Elymus 21 days after treatment with 0, 100, 200, 300 and 400 mM NaCl impacted irrigation water.

Year	VARIETY	TRT	N	S	P	K	MG	CA	NA	Cl	B	Zn	Mn	Fe	Cu	Al
			----------%----------								----------ppm----------					
1	CAWR	0	3.41	0.16	0.56	4.05	0.20	0.48	0.09	0.96	10.00	29.75	60.50	61.25	8.50	7.50
1	CAWR	100	3.48	0.20	0.47	3.26	0.26	0.77	0.70	2.36	8.50	32.50	88.00	65.25	9.00	3.75
1	CAWR	200	3.56	0.19	0.47	3.18	0.30	1.04	1.20	3.24	7.75	37.50	102.50	59.00	8.00	2.50
1	CAWR	300	3.59	0.21	0.48	3.26	0.34	1.22	2.09	4.40	8.50	39.75	100.50	55.75	7.50	3.50
1	CAWR	400	3.66	0.20	0.44	3.02	0.33	1.23	3.28	4.96	10.00	36.50	89.50	62.25	7.50	4.25
1	RBWR	0	3.01	0.17	0.43	3.79	0.17	0.36	0.06	0.92	8.50	26.75	40.50	54.25	6.25	3.25
1	RBWR	100	3.58	0.21	0.45	3.32	0.24	0.65	1.00	2.17	8.75	36.75	77.00	62.25	7.25	4.25
1	RBWR	200	3.56	0.21	0.48	3.29	0.32	1.03	1.50	3.34	8.25	40.00	95.00	66.25	7.25	5.75
1	RBWR	300	3.55	0.20	0.47	3.49	0.35	1.27	2.66	4.92	9.00	37.50	96.25	59.75	6.75	4.25
1	RBWR	400	3.55	0.20	0.45	3.48	0.33	1.16	4.83	6.37	13.00	33.50	77.00	62.75	6.75	2.75

Table A.1 (continued)

Year	VARIETY	TRT	N	S	P	K	MG	CA	NA	Cl	B	Zn	Mn	Fe	Cu	Al
			----------%----------								----------ppm----------					
1	SEWR	0	2.47	0.13	0.41	3.76	0.15	0.36	0.05	1.34	9.00	18.00	41.25	57.50	5.50	7.50
1	SEWR	100	2.71	0.16	0.42	4.00	0.22	0.65	0.46	2.61	8.25	25.75	70.00	52.75	5.75	5.25
1	SEWR	200	2.94	0.16	0.45	4.21	0.30	1.07	1.16	4.13	9.50	31.25	103.25	62.25	5.75	6.00
1	SEWR	300	3.06	0.16	0.41	4.20	0.33	1.25	1.90	4.88	10.25	32.50	90.50	61.75	5.50	3.75
1	SEWR	400	3.52	0.21	0.45	3.80	0.38	1.44	5.95	6.23	18.75	34.25	85.00	68.25	6.25	5.50
1	VAWR	0	2.74	0.14	0.46	3.70	0.18	0.41	0.07	0.85	12.50	23.75	53.00	59.75	6.00	5.50
1	VAWR	100	3.32	0.19	0.47	3.49	0.25	0.76	1.44	2.41	9.50	37.00	89.50	70.00	7.00	3.75
1	VAWR	200	3.34	0.20	0.47	3.31	0.27	0.89	1.72	3.06	9.25	38.25	102.00	70.75	7.00	5.50
1	VAWR	300	3.36	0.18	0.43	3.61	0.33	1.21	3.71	5.14	9.50	34.75	95.75	66.75	5.50	6.25
1	VAWR	400	3.61	0.20	0.48	3.52	0.30	1.16	7.27	6.28	10.75	36.50	79.25	67.50	7.00	5.25

Table A.1 (continued)

Year	VARIETY	TRT	N	S	P	K	MG	CA	NA	Cl	B	Zn	Mn	Fe	Cu	Al
			----------%----------								----------ppm----------					
2	CAWR	0	3.85	0.28	0.56	4.21	0.33	0.54	0.09	0.70	7.75	58.75	97.25	54.00	8.00	4.75
2	CAWR	100	4.30	0.28	0.61	3.94	0.39	0.68	0.20	1.53	5.25	76.75	156.25	59.75	9.00	4.25
2	CAWR	200	4.54	0.31	0.63	3.49	0.41	0.70	0.35	1.99	4.25	81.75	122.00	62.75	10.50	2.75
2	CAWR	300	4.32	0.30	0.59	3.22	0.51	0.86	0.59	2.51	3.75	84.00	136.25	69.75	9.50	3.50
2	CAWR	400	4.69	0.35	0.60	2.82	0.53	0.92	0.83	2.94	4.00	80.25	137.50	73.50	11.00	5.50
2	RBWR	0	2.95	0.23	0.45	3.61	0.28	0.39	0.04	0.61	7.00	52.50	49.25	53.50	4.75	4.50
2	RBWR	100	3.57	0.23	0.46	3.41	0.38	0.56	0.38	1.49	5.75	70.75	94.50	72.75	6.50	6.00
2	RBWR	200	2.36	0.16	0.35	2.34	0.40	0.67	1.16	2.65	5.50	70.25	100.50	56.25	4.25	5.50
2	RBWR	300	3.63	0.24	0.48	3.32	0.48	0.78	1.34	3.00	5.75	77.25	101.25	59.25	6.00	4.50
2	RBWR	400	3.67	0.24	0.52	2.88	0.54	0.96	2.21	4.02	6.50	86.25	131.00	59.75	5.75	3.75

Table A.1 (continued)

Year	VARIETY	TRT	N	S	P	K	MG	CA	NA	Cl	B	Zn	Mn	Fe	Cu	Al
			----------%----------								----------ppm----------					
2	SEWR	0	4.04	0.27	0.60	5.25	0.36	0.51	0.08	0.82	7.50	55.50	72.50	61.75	7.50	5.25
2	SEWR	100	4.10	0.26	0.53	4.59	0.36	0.55	0.23	1.70	5.00	57.50	92.25	59.00	7.75	4.50
2	SEWR	200	4.27	0.27	0.57	4.21	0.45	0.74	0.38	2.55	6.00	69.50	130.75	66.75	8.00	4.75
2	SEWR	300	4.29	0.26	0.54	3.94	0.45	0.78	0.75	3.42	5.25	71.50	129.00	67.75	8.25	6.00
2	SEWR	400	4.11	0.28	0.55	3.64	0.51	0.94	1.59	3.81	4.50	73.75	141.00	67.25	8.75	4.25
2	VAWR	0	3.50	0.27	0.58	4.18	0.32	0.44	0.05	0.48	7.50	73.25	60.75	56.50	7.50	5.25
2	VAWR	100	3.71	0.25	0.49	3.33	0.42	0.62	0.81	1.94	5.50	82.50	104.00	57.50	7.50	4.75
2	VAWR	200	3.93	0.27	0.53	3.27	0.54	0.92	1.80	3.12	6.75	95.25	133.25	59.50	8.00	6.75
2	VAWR	300	4.11	0.26	0.51	3.16	0.48	0.79	1.37	3.21	5.25	90.50	139.50	64.50	8.25	6.50
2	VAWR	400	3.84	0.28	0.50	2.93	0.60	1.08	3.44	5.17	5.50	96.50	120.50	63.75	7.50	10.25