THESIS

SALTGRASS REVEGETATION OF SALINE SOILS

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2010

COLORADO STATE UNIVERSITY

July 14, 2010

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KORY JAMES NICKELL ENTITLED SALTGRASS REVEGETATION OF SALINE SOILS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

SALTGRASS REVEGETATION OF SALINE SOILS

Saltcedar (*Tamarix* spp.) invasion into riparian areas in southwestern US, including Colorado, is threatening native biodiversity and riparian geomorphic and hydrologic processes. Great effort and resources have been invested to eliminate and control *saltcedar* invasion. However, due to salt redistributions, saltcedar-affected sites typically have high salts content at the soil surface. Ecological restoration of sites impacted by invasion (and subsequent control) of saltcedar presents technical and conceptual challenges.

Inland saltgrass (*Distichlis spicata* L. Greene) is a warm-season, rhizomatous, perennial, halophyte with worldwide distributions. It may have potential to use as a revegetation species for salinity affected soil, including saltcedar cleared areas. Therefore, the objectives of my first study are to:

1) Collect native saltgrass germplasms on riparian sites with saltcedar present along major river systems in the western US;

2) Evaluate the collections for establishment and long-term persistence in Colorado climate by determining coverage, vigor, density, and biomass over 3-4 year period.

We collected saltgrass ecotypes along major rivers in the western U.S. from 2004 to 2006. Ninety-two ecotypes were planted in 2006 and 2007 for field observation. Data obtained for this study were: establishment as indicated by saltgrass coverage, density, height, yield, and spring green-up. Data showed significant differences among saltgrass ecotypes. Vegetative coverage was correlated to plant height and density in both years' plantings. From ecotypes planted in 2006, C30, C35, C25, C32, and C2 had the fastest establishment with good persistence. In considering all data collected, ecotype C30 is best suited for revegetation purposes; C30 exhibited the fastest establishment, and it was among the ecotypes that exhibited the highest density and yield. The growth and coverage of C30 persisted over the duration of this experiment (from 2006 to 2009). From ecotypes planted in year 2007, C51, C52, C62, C70, C115, C117, C133, C134, C135, and C137 have the best promise for revegetation purposes. Information from this study can be used to further develop saltgrass for revegetation purposes.

Two experiments were conducted in the field with the objective to determine saltgrass seed germination and establishment as affected by salinity and seed treatment chemicals (Proxy and/or Thiourea). As the average soil EC salinity increased from 3.5 to 7.6 dS m^{-1}, saltgrass seed germination was not affected. However, lower germination and plot coverage were observed in plots with soil salinity at 12.4 dS m^{-1} than the control plots. Our results indicate that Proxy solution at 5 mM a.i. enhanced saltgrass seed germination better than the other treatments at all salinity levels.

The ecotypes selected in this study can be valuable to further develop saltgrass for revegetation purposes. The information on saltgrass germination as affected by

salinity and proxy treatment can be integrated into development of protocols for revegetation of saline areas.

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ACKNOWLEDGEMENTS

First and foremost, my Mom and Dad have been leaders in encouraging my continued efforts for a better future. Thank you. I would also like to thank everyone I've come in contact with in the Fort Collins community including those whom have graciously served my best interests at Colorado State University. It really has been an awesome experience. I owe a debt of gratitude to each of the faculty members I've had the pleasure of learning from.

I would like to express my gratitude to my advisor, Dr. Yaling Qian, whose inexhaustible guidance, valuable advice, support, and constructive criticism helped me greatly throughout the duration of my graduate study. I appreciate her for initiating this study and helping me to meet challenges and overcoming difficulties of, in my opinion, great magnitude. She has been more than an advisor and given unprecedented counsel toward the successful completion of this manuscript.

I owe a debt of gratitude to Dr. Koski. I was working on a golf course and had contacted him for information pertaining to grass establishments on several occasions. The work that is performed through extension is invaluable to society and cannot ever be thought about lightly if we are to remain viable as a society. The outreach performed through CSU is incredible.

Dr. Hansen has been my go to guy for all kinds of advice. I can ask him any question to which he genuinely gives good advice about. Every time I've spoken with him, I've felt as though I've left a better person, more able to handle obstacles, with confidence, which I may face on my path. His ability to relate to different personality types with ease and comfort are truly something to aspire to.

Dr. Fenwick has been a key-stone in the arch of education I have a pleasure to walk through. I came to CSU with the intent on being a Peace Corps Master's International student of which he was the program coordinator at the time. His loyalty to aiding students in successfully completing academic programs, and his stability to relate to multiple styles and levels of learning are tremendous. He's a legend.

Sarah Wilhelm has aided my research and mental capacity to handle graduate school with great dignity and without exception. She kept me hopeful that truly this manuscript would someday come to complete fruition. The job she performs is invaluable to the richer more rewarding experience each student may have when attending CSU whom have the pleasure to work with her.

Joe Cosenza has expanded my views about raccoons, bacteria, and religion alike, and enlightened my understanding toward thinking of the world in which I live.

Dana Christensen has aided my research in many ways and provided many suggestions to further develop methods and materials.

Mr. Jim zumBrunnen has patiently dedicated his valuable services in instruction to properly analyzing data. His guidance and direction toward interpretation of statistical results are invaluable. I could not have been able to achieve the type of manuscript I have without technical help provided by the statistic department.

This study was funded in part by the Bureau of Reclamation and the Colorado State Agriculture Experiment Station's Invasive Weeds program. Without generous support and contributions made by organizations, research and development would not exist.

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Chapter 1:

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Abstract

Saltgrass, native to western U.S., is a species showing promise for revegetation of saline soils. Specific traits such as rapid establishment, high plant density, and high shoot biomass production are desirable for the success of revegetation. The purpose of this study was to enhance knowledge regarding the establishment capabilities of inland saltgrass for increased habitat restoration effectiveness, and for addressing related water salvage concerns in riparian saltcedar infestations. We collected saltgrass ecotypes along major rivers in western U.S. from 2004 to 2006. Ninety-two ecotypes were planted in 2006 and 2007 for field observation. Data obtained for this study were: establishment as indicated by saltgrass coverage, density, height, yield and winter hardiness as determined by winter survival in the field. . Data showed significant differences among saltgrass ecotypes. Biomass yield was positively correlated to density and negatively correlated to plant height. From ecotypes planted in 2006, C30, C35, C25, C32, and C2 had the fastest establishment with good persistence. In considering all data collected, ecotype C30 is best suited for revegetation purposes in Colorado climate; C30 exhibited the fastest establishment, and it was among the ecotypes that exhibited the highest density, height, and yield. The growth and coverage of C30 persisted over the duration of this experiment (from 2006 to 2009). From ecotypes planted in year 2007, C51, C52, C62, C70, C115, C117, C133, C134, C135, and C137 have the best promise for revegetation purposes in

Colorado climate because of exhibited winter hardiness characteristics. Information from this study can be used to further develop saltgrass for revegetation purposes.

Introduction

Salt pollution of surface waters and ecosystems is problematic world-wide. This is mainly caused from natural mineralization processes. In arid and semi-arid environments where evapo-transpiration exceeds rainfall, salinity accumulation on soil surfaces may occur (Cardon, 2007). Human induced distribution of salts, such as the use of fertilizers, roadside de-icing salts, and using saline water for irrigation, results in the disruption of drainage systems. In addition, high water tables containing salts may also cause increased salinity levels in the root zone of plants through capillary rise in the soil (Barrett-Lennard, 2002).

Some plants are more tolerant of soil salinity than others. Well adapted, aggressive, non-native species which can out-compete more salt-sensitive native plants on soils affected by high salinity levels may become noxious. Saltcedar species (*Tamarix* spp.) are halophytic, facultative phraetophytes which can dominate competition with other plants under both wet and dry conditions. This plant was brought to the United States from Europe or Asia in the 1800's for erosion control (Vandersande et al., 2001; Brotherson and Field, 1987). During establishment, saltcedar will send a tap root into the soil profile in search of water. Upon contact with water, secondary branching of the root becomes profuse. Its roots are adventitious and have the ability to rapidly grow in moist soils. Additionally, it reproduces by seed (potentially up to 500,000 seed per mature plant per season) during an extended period of bloom from potentially late May to October (Busch and Smith, 1995).

Millions of dollars are spent each year in an attempt to control saltcedar using mechanical, chemical, and/or biological methods. It is well documented that saltcedar invasion produces undesired environmental effects in riverine and lacustrine systems (Shafroth et al., 2008). Adverse effects of saltcedar include but are not limited to: increased wildfire hazard resulting from high densities of fine woody fuel material, reduced biodiversity and wildlife habitat, modifications to riparian ecosystem function and structure, and reduced surface and groundwater return flows (Lair, 2006).

Saltcedar is believed by Ladenburger et al. (2006) to cause changes in soil chemistry (salinity and fertility islands) below canopy. This study found that surface soil beneath saltcedar canopy, when compared to soil in interspaces, had higher EC levels at the surface and lower pH levels in deeper soils. It was theorized from findings that higher salinity was caused by deposition of soluble salts with litter of saltcedar. Salts, which are taken up by roots from the water table or soil, are exuded from foliage. Other studies have also determined that when litter is shed from some halophytes such as saltcedar, the soil surface may become concentrated with salts (Brotherson and Field, 1987; Busch and Smith, 1995).

Saltcedar has displaced native species from many thousands of acres (Harms et al., 2006). Ecosystem biodiversity can be further reduced for long periods of time where salts affect the ideal growth of plants by a combination of factors relating to physiological drought or ion toxicity (Munns and Tester, 2008). Native biodiversity reduction that is caused by highly aggressive, invasive saltcedar species on saline sites has been profound.

Reported salinity levels on sites with saltcedar stands younger than 44 years old have commonly been reported in the range of 5.7 to 15 dS m^{-1} (Carmen and Brotherson, 1982; Ladenburger, 2006). Busch and Smith (1995) noted that salinity levels along the Colorado River were 12.8 dS m^{-1} to a soil depth of 90 cm. Due to high salt levels, sites cleared of saltcedar may be difficult to restore to a pre-existing ecological state (D'Antonio and Meyerson, 2002), thus restoration should be objective based (Dufour and Piegay, 2009; Shafroth et al., 2008). An over-arching goal of restoring sites cleared of saltcedar, especially soils which are saline, is to shorten the time of a soil's bare period by establishing a diverse mixture of perennial species. Bay and Sher (2008) note that active revegetation following removal of saltcedar can greatly assist native plant community recovery and aid in long-term saltcedar control.

Saltgrass (Distichlis spicata (L.) Greene) has potential to be used in revegetation mixes on sites cleared of saltcedar (Lair and Wynn, 2002). Saltgrass is a warm-season, rhizomatous, perennial species able to withstand drought, extended periods of low available oxygen levels, and temperature extremes (Alshammary et al., 2004; Warren and Gould, 1982). It is native to Western U.S. and is able to tolerate many other stresses including: wear, compaction, and high salinity (Kopec and Marcum, 2001; Qian et al., 2007). For example, saltgrass has been shown to tolerate salts at approximately 20-50 dS m^{-1} (Marcum et al., 2007; Marcum et al., 2005; Qian et al., 2007), and is documented to remain green during the hottest parts of the summer when all other grass species around it have gone dormant due to heat and drought stress (Hansen et al., 1976). It is a member of the Poaceae family, and can be found from South America to Canada and across the U.S. It grows in sandy to heavy clay soils and is adapted to a wide range of pH levels which allows for it to be one of the most widespread and common halophytes in the U.S. (Ungar, 1974).

Saltgrass species comprise large genetic pools and have been able to develop many phenotypic and morphologic traits allowing for adaptation and increased survivability in nature (Christensen, Personal Communication, 2009). In nature, the primary mode of regeneration for saltgrass is by means of sharp, scaly rhizomes which can penetrate through high strength shales or deflocculated, sodic soils thus enhancing soil water infiltration (Marcum et al., 2007). In Australia, it has been reported through extensive soil sampling and landholder observations over the course of eight years in a saline discharge zone that the growth of saltgrass improves the soil chemical and physical conditions (Sargeant et al., 2008).

Selection of vigorous lines of saltgrass can increase the success of ecological restoration on many salt affected sites in addition to reducing re-infestation potentials of noxious weeds (Taylor and McDaniel, 2004). It has been known for many years that there are large differences in characteristics between ecotypes within a plant species. The genetic variability within a species is not only a valuable tool for studying mechanisms of tolerance to different factors, but also an important basis for selecting and breeding species (Marschner, 1995; Wang et al., 2001).

Saltgrass is commonly referred to as either seashore saltgrass (Distichlis spicata (L.) Greene var. spicata) or desert (inland) saltgrass (Distichlis spicata (L.) Greene var. stricta); however, both are members of the same species (Ram et al., 2004). Saltgrass germplasm lines display significant differences in regards to density, cold hardiness, salinity, and drought tolerance

(Marcum et al., 2005; Marcum et al., 2007; Qian et al., 2007; Rukavina et al., 2008; Shahba et al., 2003a). However, for reclamation purposes, the ability to rapidly establish while maintaining persistence in subsequent years growth is of upmost consideration. High density, fast growth, and good vigor can allow for better competition with potential (re)infestations of noxious species via root sprouts or seed germination from occurrence on site. In Colorado climate (USDA hardiness zone: 4), cold temperatures may be the most injurious environmental condition for a C4 grass species. Additionally, yield and height are important vegetative traits for habitat restoration for wildlife, surface cover of soil for conservation purposes, and/or for advantages associated with competition for light, water, nutrients, and space with other plant species.

Therefore, the objectives of this research are to:

1) Collect native saltgrass germplasms on riparian sites with saltcedar present along major river systems in the western US;

2) Evaluate the collections for establishment;

3) Evaluate each collection for its long-term persistence in Colorado climate by determining coverage, vigor, density, and biomass over 3-4 year period.

The goal is to select saltgrass ecotypes with superior establishment characteristics and long-term persistence for revegetation. It is hypothesized that significant differences will be observed between different saltgrass ecotypes for rate of spread over a defined area as well as the density of growth within that defined area.

Methods and Materials

PLANT COLLECTION AND GROWTH CONDITIONS

Saltgrass ecotypes were collected throughout southwest United States. In 2004, 19 ecotypes were collected, 36 in 2005 and 85 in 2006. Most ecotypes were collected along the major waterways and tributaries of the: Colorado, Rio Grande, Pecos, Arkansas, Canadian, and Red rivers. Further, the states which ecotypes were sampled from include: Colorado, Texas, New Mexico, Arizona, inland California, Nevada, and Utah (Tables 1 and 2). Ecotype samples were collected mainly as rhizomes; however seeds were also obtained for two ecotypes in planting year 2007 (C140 & C44B in Table 2). These collected materials were brought to CSU greenhouse facility to be established in 6.5 cm diameter by 32 cm deep pots filled with greenhouse mix media.

When pot surfaces were completely filled by saltgrass shoots, the ecotypes were planted for field observation at the Horticulture Research Center of Colorado State University located north of Fort Collins, CO. Planting times occurred during the month of May in years 2006 and 2007. The soil at the research center is a Nunn clay loam (fine, smectitic, mesic Aridic Argiustoll). The average soil salinity was 3.8 dS m^{-1} and soil pH was about 7.8. The average first frost date typically comes around September 22 in Fort Collins.

In May of 2006, individual plugs of 48 ecotypes were planted in the center of 2.32 m X 2.32 m plots and in May of 2007, ninety two different ecotypes were planted in the center of 1.5 m X 1.5 m plots. At all plot edges, 30 cm buffer space between plots was maintained by applying glyphosate monthly or as needed to prevent potential contamination of adjacent plots. Weeding was carried out by hand pulling non-saltgrass plants from each plot. Immediately after planting, saltgrass plots were hand watered so that contiguous soils became saturated. In planting year 2006, a linear overhead irrigation sprinkler provided water at a rate of 61 mm the first week of establishment, and then 40.6 mm each following week for the next three weeks. For ecotypes planted in 2007, 40.6 mm of water were applied to the field during the months of May and again in June. The rate of water was applied at approximately 20.3 mm per irrigation. Additionally, no fertilizers were applied to any plot and the field was left un-mowed during the first 2 years for this study.

PARAMETER RATINGS

Characteristics evaluated for this study include establishment (plot coverage) and density for revegetation purposes, fall color retention and percent spring green-up for persistence, as well as height and aboveground biomass production (yield) for habitat restoration purposes.

Ecotypes planted in 2006 were visually rated for percent of plot coverage using a 2.3 m X 2.3 m PVC frame placed on center of each plot. Ecotypes planted in 2007 were rated using a 1.5 m X 1.5 m PVC frame. For planting year 2006 ecotypes, plot coverage observations were recorded once at the end of the 2006 growing season and once in May, 2007. Ecotypes planted in 2007 were recorded for plot coverage at the end of 2007 growing season. In 2008, all ecotypes were rated every 3 weeks starting in May. In 2009, all ecotypes were again observed for coverage throughout the summer a total of three times (May, June, and August).

Density ratings were based on a scale of 1-5. A rating of 1 indicated very thin turf with much bare soil visible (~95%) between individual plants. A rating of 3 had medium density (~40-60% visible bare soil showing between actively growing plants) and a rating of 5 indicated individual plants growing very close together with less than 5% bare soil visible. Density ratings were recorded 3 times throughout the summer of 2008 as well as 2009.

Beginning early summer, the percent area that showed spring green-up was recorded to determine persistence. Ecotypes planted in 2006 were rated for fall color retention in October 2006 based on a scale of 1-5 where 1 denoted completely brown, dormant turf, a rating of 2.5 was recorded for 50% dormant turf, and a rating of 5 was completely green with no visible signs of dormancy. In September 2007, ecotypes planted in 2007 were rated for dormancy based on a 1-9 scale where 1 denoted completely brown dormant turf and a rating of 9 signified completely green turf with no signs of dormancy. During 2008, all ecotypes were rated for fall color retention on 3 dates based on the 1-5 scale previously described. In 2009, all ecotypes were mowed and dormant biomass removed to more accurately determine percent green-up that had occurred.

During summer 2008 and 2009, soil surface to leaf tip length measurements of fully mature plants were taken in 3 places within each established plot. Measurements obtained from each plot were averaged and recorded. Yield was determined by removing biomass at the soil surface from a 0.09 m^2 PVC framed area. Biomass was dried for 24 hours at 60°C. Dry weights were recorded and converted to Mg ha^{-1}.

STATISTICAL ANALYSIS

The experiment was arranged in a randomized complete block experimental design with 2 replications. Data were analyzed by planting year using SAS software (version 9.1; SAS Institute, Cary, NC). Cover data were transformed using arcsine. Mixed procedure was run with random replicate and repeating date as a fixed effect to determine correlation coefficient for a linear fit model in analyzing establishment. Utilizing Tukey's honest significant difference with the Glimmix procedure (generalized linear mixed model), separation of means were determined for percent cover, density, height, and yield. Pearson product moment analysis was conducted to determine the relationship of correlation coefficients for plot cover, density, height, and yield variables.

Results and Discussion

Some samples did not survive the climatic conditions at the research site. In addition, some plots were determined to be other grass species due to misidentification at the time of collection which were excluded from data analysis. Consequently, data consisted of observations for 32 ecotypes in planting year 2006 (Table 1) and 60 ecotypes in planting year 2007 (Table 2).

Planting Year 2006

Cover, Establishment, and Winter Hardiness

Ecotypes planted in 2006 differed in percent cover(P=0.03). Rapid establishment during the first year is important to eliminate weeds from colonizing a site. The ecotype with the best cover at the end of the growing season in 2006 and beginning of 2007 was C30. C30 remained in the top statistical category during all dates of observation for cover throughout the study. In 2006, ecotypes not statistically different in cover from C30 were: C41, C32, C53, C13, C35, C25, C22, C45, C31, C28, and C2. In May 2007, ecotypes not different in cover from C30 were: C35, C25, C2, and C32.. Therefore, ecotypes C30, C35, C25, C2, and C32 had fast establishment and good persistence. Those ecotypes (C41, C53, C13, C22, C45, C31, and C28) which were in the top statistical category in 2006, yet not in 2007, were affected by winter injury.

From evidence of other studies, the winter minimum temperature during each year may be the cause of cold injury (Rukavina et al., 2007; Shahba et al., 2003a; Shahba et al., 2003b).

More specifically, in a study conducted by Rukavina et al. (2007), it was observed that ecotypes of saltgrass collected from zone 4 had an average LT_{50} (that is lethal temperature at which 50% of rhizomes died) at -17.2 C. Schwarz and Reaney (1989) determined from a controlled environmental study that inland saltgrass collected from zones 2 and 5, survived to -35 C. In the same study, rhizomes exposed to temperatures above -25 C were able to provide water and nutrients for crowns to aid with winter hardening process. Soil insulates rhizomes from temperature extremes as opposed to the aboveground biomass. For our study, the minimum soil temperature was approximately -13° C in the top 5 cm of soil (data not shown).

However, the ecotypes evaluated in our study were collected from USDA Hardiness zone 5-10. Therefore, our study suggests that ecotypes collected from warmer hardiness zones are not able to tolerate as cold of temperatures as those collected from cooler hardiness zones.

During the growing season of 2006, 12 ecotypes were in the top statistical category. From November 2006 to February 2007, atmospheric temperatures dropped to below -20 C on 12 dates. The daily minimum temperatures in 2006 began to drop below zero in mid-September. Additionally, daily minimum temperatures can clearly be seen to drop to below -5 in mid-October, with the low temperature during 2006 reaching -29.22 C on November 30 (Figure 2). This is the earliest date in which temperature dropped to below -25 C in this four year study. In observation year 2007, only five of the 12 ecotypes in the top statistical category in 2006, remained in the top category in 2007. In 2008, 21 ecotypes were in the top statistical category. Saltgrass cold tolerance increased as the field plots matured. Plots in 2008 were established with sufficient plant material to buffer the temperature extreme occurrences.

Glimmix analysis of cover data in 2008 and 2009 showed that C26 was rated highest in plot coverage for both of these years. Ecotypes that were not different from C26 in 2008 were: C35, C41, C30, C25, C6, C22, C43, C2, C38, C32, C28, C3, C40, C18, C1, C9, C19, C8, C13, C11, and C10. In comparison, 2009 cover data of ecotypes rated in the top statistical category were: C26, C2, C32, C30, C44, C25, C3, C35, C41, C1, C22, C38, C28, C6, C46, C31, C8, C43, C9, C27, C53, C40, and C47. Therefore, for rapid establishment and persistence, ecotypes C30, C35, C25, C32, and C2 displayed the most consistent high levels of coverage despite seasonal variability. However, further testing is needed to screen for salinity tolerance amongst these ecotypes for potential use in highly saline areas as are commonly associated with saltcedar cleared sites.

Density

The ecotype rated highest in density in 2006 planting year was ecotype C35, although C1, C26, C30, C44, C41, C28, C32, C29, C46, C38, C22, C27, C31, C25, C3, C12, C2, C11, C8 were not different from C35 (Table 4). This is 25 of 32 ecotypes evaluated that were not different from each other. Tukey's honest significant difference test was used for ecotype mean separations. This type of analysis is good to use when, for example, evaluation of a species with large genetic diversity (Christensen, personal communication, 2008) is being carried out. A majority of germplasm lines collected for planting year 2006 are statistically acceptable in this framework. Also, 2006 planting year data showed correlation of cover to density with R^2 value of 0.35 in 2008 and 0.82 in 2009 (both years $P<0.0001$; Table 5).

Height and Yield

In June 2008, planting year 2006 ecotypes height ranged from 19.1 (C46) cm to 39.1 (C43) cm in June whereas in July 2009, ecotype height ranged from 33.4 cm (C40) to 59.2 cm (C8). Ecotypes not different in height from C43 in observation year 2008 were: C9, C8, C19, C40, C6, C10, C41, C30, C3, C32, C18, C2, C26, and C53. In July 2009, ecotypes that were not different from C8 were: C18, C9, C3, C19, C41, C12, C13, C11, C32, C1, C16, C53, C10, C47, C31, C22, C43, C2, C35, C29, C28, C45, C30, C26, C46, C27, and C6 (Table 4). Ecotypes C30, C35, and C32 were among 2009 tallest ecotypes. For turfgrass evaluations, shorter ecotypes would be desired. Ecotypes such as C25, C38, C40, and C44 were of short stature and also had high density values.

While no significant correlation could be drawn between height and density in observation year 2008, density and height had an intermediately negative correlation from observation year 2009 data. Oomes (1992) suggests that because taller species intercept more light, density is reduced despite comparable levels of dry matter production for shorter ecotypes with higher density values. Our correlation analysis from 2009 data agrees with this statement as height is negatively correlated to density while density correlated strongly to yield (Table 5).

Biomass yield is becoming more and more important as the United States strives to make cellulosic biofuel a viable energy option. For planting year 2006, ecotype yields ranged from 4.5 (C25) to 11.9 Mg ha^{-1} (C1) in 2008, whereas in 2009 yields ranged from 5.2 (C19) to 16.5 Mg ha^{-1} (C3). In comparison, to show the value of other potential revegetation warm-season grasses under three irrigation regimes, Haskell sideoats grama has yielded 2.5, 5.6, and 11 Mg ha^{-1} under limited, moderate, and full irrigation regimes after two years of observation. In the same study, Blackwell switchgrass produced 3.1, 5.6, and 11 Mg ha^{-1}, and Texoka buffalograss yielded 1.1, 4.9, and 9.2 Mg ha^{-1} (Buttrey et al., 2009). In 2008, density was positively correlated to yield at $R^2 = 0.50$ (p=.0037) and in 2009 at $R^2 = 0.77$ (p<0.0001). Ecotypes that had higher yield in 2008 than the others were: C1, C28, C8, C30, C41, C44, C26, C3, and C6. The ecotype that had the highest yield in 2009 was C3; however, C44, C27, C30, C46, C31, C35, C38, C41, C28, C43, and C1 were not different from C3 in yield (Table 3). Late season heavy precipitation in 2008 and early season heavy precipitation in 2009 are believed to be strong influencing factors of height and yield. In 2009, density mean of data increased by rating of 0.5 from 2008 data and average yield of all accessions increased 3.2 Mg ha^{-1} when compared to yield data collected in 2008

In considering all data collected for accessions included in 2006 planting, ecotype C30 appears best suited for revegetation purpose. C30 exhibited the fastest establishment, and it was among the ecotypes that exhibited the highest density, height, and yield. Additionally, the growth and coverage of C30 persisted over the duration of this experiment (from 2006 to 2009).

Planting Year 2007

Cover, Establishment, and Winter Hardiness

Ecotypes planted in 2007 differed in percent cover at P<0.0001.

The ecotype that exhibited best cover rating in 2007 for establishment was C102B. Ecotypes which were not different for cover ratings from C102B were: C129, C109A, C52, C102C, C94, and C135. However, of interesting note is the fact that each of the ecotypes that had greatest establishment during growing season 2007 suffered severe injury during the winter of 2007/2008. The ecotypes that exhibited the best coverage in May 2008 were C115, C133, and C52. However C51, C62, C70, C117, C134, C135, and C137 were not different from C115. In

2009, 52 of the 61 ecotypes observed were not different from each other in percent cover of frame.

Hardening of tissue for winter survival has been documented to occur from mid-September to mid-December for northern saltgrass lines. Northern ecotypes show dormancy signs 2-4 weeks earlier than southern ecotypes (*Shahba* et al., 2003a). While cold temperatures started mid November in 2007, most ecotypes of planting year 2007 experienced some level of winter injury even though a wide range of collection site hardiness zones were represented. The only ecotypes which seemed to gain cover area in 2008 spring green-up data were: C115, C62, C51, C112, C116, C133, and C140. All of these ecotypes were collected from USDA hardiness zones 4, 5, or 6 except C133 which was collected from hardiness zone 8. During winter 2007/2008, ecotypes collected from hardiness zones 9 and 10 had mean percent cover loss of approximately -21% while all other collection site hardiness zones (3-8) were approximately -2%. This supports previous findings that saltgrass cold hardiness is associated with each ecotype's origin, i.e. the accessions collected from southern climates would have poor cold hardiness. However, for our study, correlation of hardiness zone to color retention rating was insignificant while color retention rating to cover was intermediately negative (R^2= -0.31) inferring that the greater fall color retention may result in low plot coverage. It is believed that some ecotypes of saltgrass acclimate to cold temperatures earlier in the autumn because of inherited tolerance mechanisms (Rukavina et al., 2007; Shahba et al., 2003a; Shahba et al., 2003b).

Some ecotypes from planting year 2007, such as C102B, C102C, and C109A, showed high vigor initially during the establishment period, yet showed winter damage and limited expansion of cover in subsequent year's observations. All three of these ecotypes were collected from hardiness zone 9 or 10. These collections may be very valuable for revegetation use in warmer climate zone, but their potential use in cold climate region, such as Colorado, is limited.

While ecotypes C52 and C135 displayed coverage that was not different from the highest rated ecotype in each observed year, ecotypes C115, C133, C51, C62, C70, C117, C134 and C137 were in the top statistical category for coverage in May 2008 as well as in concurrent ratings indicating good establishment and more tolerance to climactic variables than the others.

Density

Ecotypes not significantly different from ecotype C121 (rated with the highest density) in 2007 planting year were: C55, C75, C117, C71, C83, C135, C95, C127, C70, C118, C133, C62, C116, C51, C63, C137, C102A, C115, C54, C102B, C52, C94, C87, C129, C44B, C61, C78, C134, C140, C68, C67, C93, C92, C111, C130, C102C, C138, C139, C120, C85, C79, C53, and C99 (Table 4). Planting year 2007 data showed correlations of density to cover with $R^2 = 0.47$ in 2008 (p=0.0002) and R^2= 0.51 in 2009 (p<0.0001).

Height and Yield

For planting year 2007, ecotype height ranged from 17.7 cm (C118) to 35.6 cm (C94) in June 2008 whereas during July 2009, ecotype height ranged from 23.0 cm (C117) to 57.9 cm (C60). The ecotypes which were not different from C94 in June 2008 were: C61, C95, C132, C135, C93, C130, C129, C85, C53, C100, C70, C78, C60, C62, C63, C99 and C138. However, in July 2009, ecotypes which were not different from C60 were: C103A, C129, C93, C71, C139, C94, C78, C130, C85, C62, C120, C95, C135, C87, C63, C132, C98, C61, C54, and C103B (Table 7). Shorter ecotypes would be desired in turfgrass evaluation. Ecotypes such as C68, C75, C115, C117, C118, and CDD were of short stature and also had high density values. For purposes of wildlife forage value, taller ecotypes may be more desirable.

Ecotype that had the highest yield in 2008 was C75. However, C102A, C70, C71, C107A, C120, C60, C130, C102B, C102C, C95, C133, C55, C68, C63, C83, and C135 were not different from C75 (Table 4). For year 2008, ecotype yields ranged from 3.4 to 11.4 Mg ha^{-1}, whereas in 2009 yields ranged from 5 to 15.2 Mg ha^{-1}. Ecotype that had the highest yield in 2009 was C121, although C63, C55, C70, C67, C137, C133, C134, C83, C68, C111, C85, C115, C62, C44B, C78, C129, C102A, C139, C75, C135, C112, C79, C140, C66, C118, C95, C103B, C130, C61, C117, C132, C53, C102B, and CDD were not different from C121 (Table 4).

Biomass yield is significantly correlated to density with $R^2 = 0.67$ in 2008 and $R^2 = 0.46$ in 2009. From 2008 to 2009, the density increased by a rating of 0.7, which equated to an increase in yield by 4 Mg ha^{-1} (Table 7).

Both height and yield increased from 2008 to 2009 in both planting years 2006 and 2007. The higher yield and density observed in 2009 were likely a result of the higher than average precipitation occurred during April and June 2009. 2008 rainfall was 75% (15.1 cm) of the historic monthly average from April to July, while 2009 rainfall was 136% (27.3 cm). From 1957 to 2001, the average rainfall from April through July was 20.1 cm (Figure 1).

Conclusions

In this study, 3-4 year observations were carried out for different saltgrass ecotypes collected throughout Southwest U.S. Saltgrass vegetative coverage and density were significantly correlated to one another, as was density to yield in both planting years. Therefore, density is an important trait to observe. Higher density may be a cause or a result of shorter ecotypes, while higher density may also be associated with higher yielding plants in addition to higher cover of vegetated area. For ecotypes planted in 2006, ecotypes C30, C35, C25, C32, and C2 had faster establishment and good persistence. These ecotypes are suitable for revegetation purposes. In considering all data collected, ecotype C30 is best suited for revegetation purpose in Northern Colorado climate (Picture 2). C30 exhibited the fastest establishment, and it was among the ecotypes that exhibited the highest density and yield. The growth and coverage of C30 persisted over the duration of this experiment (from 2006 to 2009).

From ecotypes planted in 2007, C102B, C102C, and C109A showed exceedingly high vigor initially during the establishment period, yet showed winter damage and limited expansion of cover in concurrent year's observations. These ecotypes were collected from hardiness zone 9 or 10. These collections may be very valuable for revegetation use in warmer climate zone, but their potential use in cold climate region, such as Colorado, is limited. However, ecotypes C51, C52, C62, C70, C115, C117, C133, C134, C135, and C137 show the best promise for revegetation purposes. More research is needed in order to further screen ecotypes for salinity as well as determine suitability in other climates such as the southwestern U.S.

Table 1.1: Collection site and soil characteristics of 2006 planted ecotypes.

CSU#	Collection site	Collection site soil type and/or miscellaneous notes
C1	Lake Meredith recreation area near the Canadian River, TX	Silt loam alluvium; high pH
C2	Lake Meredith recreation area, Potter County, TX	Silt loam alluvium; high pH
C3	Randall County, TX	Loamy soil; high pH
C6	Caballo Reservior, N.M.	Tight clay; high water table
C8	Boulder City, NV	NA
C9	Boulder City, NV	NA
C10	Boulder City, NV	Coarse, bigger plants
C11	Boulder City, NV	Coarse, bigger plants
C12	Boulder City, NV	NA
C13	Boulder City, NV	NA
C16	Boulder City, NV	NA
C18	Boulder City, NV	NA
C19	Boulder City, NV	NA
C22	Truth of Consequence, N.M.	Heavy clay; shallow water table (<1ft)
C25	South of Polomas Meadow, N.M.	Dry and clayey soil
C26	South of Polomas Meadow, N.M.	Compacted ground; dwarf plants
C27	"Bosque Del Apache" Wildlife Refuge, N.M.	Sandy silt loam; white salt crust present on the surface
C28	"Bosque Del Apache" Wildlife Refuge, N.M.	Same as C27
C29	"Bosque Del Apache" Wildlife Refuge, N.M.	NA
C30	"Bosque Del Apache" Wildlife Refuge, N.M.	Dry, highly saline soil; dense canopy
C31	"Bosque Del Apache" Wildlife Refuge, N.M.	Fine textured leaves
C32	Sevilleta National Wildlife Refuge, N.M.	NA
C35	Soccoro County, N.M.	Sandy loam soil
C38	Capitan, N.M.	Collected from a drainage ditch
C40	Artesia, N.M.	Soil is very dry
C41	Pecos River, NM	NA
C43	Lake Avalon near Carlsbad, NM	Tall, male ecotype
C44	Pecos River in N.M.	Turf looking
C45	Pecos River at Santa Rosa site, TX	Very rocky, sandy loam
C46	Not Available	Poor density
C47	Canadian River at Maxwell, NM	Dry, rocky soil
C53	Arkansas River, TX	Dense stand of fine textured plants

Table 1.2: Collection site and soils characteristics of 2007 planted ecotypes.

BOR #	Collection site	Collection site soil type and/or miscellaneous notes
C51	Goose Lake in Modoc County, CA	Saline lake bed sediments, high water table, and poor drainage
C52	Goose Lake in Modoc County, CA	Stratified soils that are poorly drained and salt-affected
C53	Arkansas River, TX	Fine textured male plants
C54	Salt Lake, UT	NA
C55	Grand Junction, CO	Soft and good turf type
C60	Rocky Ford, CO	Fine sandy silt
C61	Rocky Ford, CO	Fine sandy silt
C62	Rocky Ford, CO	Fine sandy silt
C63	Rocky Ford , CO	Fine sandy silt; prominent seed head production
C66	Blue Lake near Las Animas, CO	Clay soil
C67	Blue Lake near Las Animas, CO	Clay soil
C68	Gagerty Creek, CO	Clay soil
C70	John Martin Reservoir State Wildlife Area, CO	Sand and rock
C71	John Martin Reservoir State Wildlife Area, CO	Sand and rock
C75	Arkansas River, CO	Clay loam
C76	Arkansas River at Fowler, CO	Fine, silty, sand loam
C78	Arkansas River at Fort Lyons, CO	Fine sand with some silt
C79	Arkansas River at Fowler, CO	Silt loam with rocks
C83	Arkansas River at Fowler, CO	Fine, silt loam
C85	Arkansas River at Avondale near Pueblo, CO	Fine silt with many rocks
C87	Arkansas River at Canyon City, CO	NA
C90	Arkansas River at Florence, TX	Very rocky, moist, disturbed loam
C92	Colorado River at Palisade, CO	NA
C93	Green River, UT	Disturbed silt loam
C94	Green River, UT	Disturbed silt loam
C95	Sevier River, UT	Clay soil
C96	Sevier River at Joseph, UT	Very dry, clay loam
C98	South of Cedar City, UT	Moist soil
C99	Colorado River at Laughlin, NV	Clay loam
C100	Lake Havasu City, AZ	Sandy loam
C104	Laguna Dam at Mittry Lake, AZ	Silt loam
C111	Poudre River trail, CO	Dry, rocky, silt loam
C112	Poudre River trail, CO	Dry, rocky, silt loam
C115	Poudre River trail, CO	NA
C116	Poudre River trail, CO	Dry, rocky soil
C117	Poudre River trail, CO	Soil high in limestone
C118	Poudre River trail, CO	Compacted, dry soil
C120	Rio Grande River at El Paso, TX	Water table about 3 feet

C121	Rio Grande River at El Paso, TX	Water table about 3 feet
C127	Rio Grande River at Anthony, TX	Sandy soil
C129	Rio Grande River at Vado, TX	Compacted, dry soil
C130	Rio Grande River at Mesilla, TX	Sandy soil
C132	Rio Grande River at Las Cruces, TX	Compacted, dry soil
C133	Las Cruces, NM	Very rocky, dry irrigation ditch
C134	Las Cruces, NM	Sandy soil
C135	Rio Grande River, CO	Clay soil
C137	Rio Grande River at Radium Springs, N.M.	Very dense growth
C138	Rio Grande River at Radium Springs, N.M.	Very dense growth
C139	Rio Grande River at Radium Springs, N.M.	Dry, sandy soil
C140	Rio Grande River at Radium Springs, N.M.	Dry, sandy soil; only seed heads collected
C102A	Bill Williams Reservoir, AZ	Fine, sand loam
C102B	Bill Williams Reservoir, AZ	Fine, sand loam
C102C	Bill Williams Reservoir, AZ	Fine, sand loam
C103A	Laguna Dam at Mittry Lake, AZ	Silty soil
C103B	Laguna Dam at Mittry Lake, AZ	Silty soil
C106B	Yuma Proving Grounds, AZ	Silty, sandy, moist soil
C107A	Yuma Proving Grounds, AZ	Silty, sandy, moist soil
C109A	Yuma Proving Grounds, AZ	Dry, fine, silt loam
C44B	Pecos River, NM	Only seed heads collected
CDD	NA	NA

Table 1.3: Planting year 2006 establishment and persistence traits observed according to collection site.

Ecotype	Coverage Ratings (% turf cover of 2.32 m^2 frame)				USDA Hardiness Zone
CSU#	Sept. 2006 cover	May 2007 cover	2008 L.S. Means	2009 L.S. Means	1 (cooler)–10 (warmer)
C1	14	19.5	0.9*	1.5*	6
C2	15.3*	27.8*	1.0*	1.6*	6
C3	10.5	17.8	1.0*	1.5*	6
C6	10.8	21	1.1*	1.4*	6
C8	14.3	15.5	0.9*	1.3*	8
C9	9.3	9.5	0.9*	1.3*	8
C10	10.5	10	0.8*	1.0	8
C11	13.3	19	0.8*	1.1	8
C12	12.8	13.3	0.5	1.1	8
C13	19.5*	20.3	0.8*	1.0	8
C16	12.3	16	0.6	1.0	8
C18	10.8	15.5	0.9*	0.9	8
C19	13	20	0.9*	1.0	8
C22	17*	19	1.1*	1.4*	6
C25	18.8*	30.3*	1.1*	1.5*	6
C26	14	20.3	1.2*	1.6*	6
C27	13.3	15	0.7	1.2*	6
C28	15.3*	18.3	1.0*	1.4*	6
C29	9.8	7	0.5	1.2	6
C30	25*	36.8*	1.1*	1.6*	6
C31	15.8*	16.5	0.7	1.3*	6
C32	21.5*	26.8*	1.0*	1.6*	6
C35	18.8*	31.5*	1.2*	1.5*	6
C38	7.8	8	1.0*	1.4*	6
C40	13	16.8	0.9*	1.2*	6
C41	22.3*	16.8	1.2*	1.5*	7
C43	14	17	1.1*	1.3*	7
C44	9.5	7.5	0.6	1.6*	9
C45	16*	16.8	0.6	1.1	9
C46	13.8	15.3	0.5	1.4*	Uk
C47	14.3	14.3	0.5	1.2*	5
C53	20.5*	20	0.7	1.2*	Uk
M.S.D.	10.3	13.4	.	.	

*** indicates ratings not significantly different from highest rating within that column.**

Table 1.4: Traits for habitat value and competition with other species: observations for planting year 2006 ecotypes.

Ecotype	Average Density (1-5 scale)		Yield (Mg ha^{-1})		Height (cm)	
CSU #	**2008**	**2009**	**2008**	**2009**	**Jun-08**	**Jul-09**
C1	3.7*	3.7*	11.9*	11.3*	28.3	51.8*
C2	3.0*	3.7*	5.4	9.02	31.1*	49.1*
C3	2.8	3.9*	8.2*	16.5*	31.4*	55.2*
C6	3.0*	3.4	7.9*	9.1	33.7*	42.2*
C8	3.2*	3.3	11.0*	7.4	36.5*	59.2*
C9	3.0*	3.3	4.6	9.5	37.5*	57.9*
C10	3.0*	3.1	5.7	8.8	33.3*	50.7*
C11	3.3*	3.0	6.8	8.5	25.1	52.8*
C12	3.2*	3.2	7.2	7.5	23.8	54.1*
C13	2.5	3.2	7.5	10.8	24.1	53.6*
C16	3.0*	3.3	7.0	10.9	25.7	51.4*
C18	3.5*	2.5	6.4	7.2	31.1*	58.7*
C19	2.7	2.7	7.0	5.2	35.9*	54.7*
C22	3.2*	3.6*	7.3	9.3	29.5	49.7*
C25	3.0*	3.6*	4.5	7.8	26.4	34.9
C26	3.5*	3.8*	8.8*	10.6	30.8*	45.8*
C27	2.8	4.0*	6.0	13.8*	27.3	44.2*
C28	3.5*	3.5	11.5*	12.2*	26.7	46.7*
C29	3.5*	3.3	7.2	9.0	23.2	46.7*
C30	3.3*	4.0*	9.1*	13.5*	32.1*	46.0*
C31	3.0*	3.7*	7.7	13.0*	25.1	49.8*
C32	3.0*	4.0*	7.5	11.0	31.1*	33.4
C35	3.5*	4.1*	6.4	12.8*	24.8	46.9*
C38	3.0*	3.8*	5.5	12.5*	26.0	40.8
C40	3.0*	3.4*	6.1	9.5	35.2*	33.4
C41	3.3*	3.8*	8.9*	12.4*	32.1*	54.4*
C43	3.0*	3.3	7.4	11.6*	39.1*	49.4*
C44	3.0*	4.2*	8.8*	16.0*	20.3	40.0
C45	2.7	3.4*	5.2	8.7	28.9	46.5*
C46	3.0*	3.9*	7.3	13.4*	19.1	45.2*
C47	2.3	3.7*	5.3	10.8	21.6	50.4*
C53	2.8	3.4	5.4	7.9	35.9*	51.2*
Mean	**3.0**	**3.5**	**7.3**	**10.5**	**29.1**	**49.2**

*** indicates ratings not significantly different from highest rating within that column.**

Table 1.5: Planting year 2006 correlation of yield, height, density, and cover.

	2008 Pearson Correlation Coefficients			2009 Pearson Correlation coefficients		
	Height	Density	Cover	Height	Density	Cover
Yield	0.03	0.50*	0.22	-0.23	0.77*	0.55*
Height		0.03	0.51*		-0.43*	-0.37*
Density			0.35*			0.82*

* indicates significance at p=0.05

Table 1.6: Planting year 2007 establishment and persistence characteristics observed from 2007-2008 and estimated in 2009.

Ecotype	Coverage Ratings (% turf cover of 1.5 m^2 frame)				USDA Hardiness Zone
CSU #	Oct. 2007	May 2008	Aug. 2008	2009 L.S. Means	1 (warmer) - 10 (cooler)
C51	18	18.5*	92.5*	1.6*	6
C52	33*	21.3*	89.5*	1.6*	6
C53	16.5	15.5	62.5	1.6*	UK
C54	15	10	52.5	1.4*	5
C55	13	14.5	77.5*	1.5*	5
C60	11	10.5	40	1.6*	5
C61	16	15.3	62.5	1.5*	5
C62	17	19.5*	80*	1.5*	5
C63	12	13.3	57.5	1.5*	5
C66	8	6	32.5	1.4*	5
C67	16	14.8	45	1.6*	5
C68	11.5	9.3	40	1.2*	5
C70	20	18.8*	80*	1.6*	5
C71	20	11.5	70*	1.6*	5
C75	17.5	16.3	45	1.4*	5
C76	10.5	5.5	42.5	1.3*	5
C78	19.5	16.8	50	1.6*	4
C79	14	12	37.5	1.5*	5
C83	16	11	52.5	1.6*	5
C85	16	11.5	50	1.4*	5
C87	16.5	17.3	65	1.6*	3
C90	16	10.5	35	1.4*	3
C92	17	16.3	55	1.4*	5
C93	12	11	47.5	1.4*	5
C94	31*	15	75*	1.5*	5
C95	17.5	14.8	47.5	1.6*	4
C96	10	7	31	1.0	4
C98	12.5	10.8	52.5	1.4*	4
C99	15.5	15.3	70*	1.5*	8
C100	16.5	10.5	47.5	1.6*	10
C104	24	3.3	35	1.0	9
C111	18.5	16	55	1.3*	4
C112	13	14.3	42.5	1.0	4
C115	20	26*	77.5*	1.6*	4
C116	12.5	14.3	47.5	1.2*	4
C117	19	17.8*	90*	1.6*	4

C118	13.5	14.3	35	1.2*	4
C120	15	13.5	60	1.5*	8
C121	18	15.5	75*	1.5*	8
C127	14.5	15	60	1.4*	8
C129	38.5*	12.5	79.5*	1.6*	6
C130	17.5	8.3	55	1.4*	6
C132	12	6.8	37.5	1.3*	8
C133	21.5	24.3*	87.5*	1.6*	8
C134	20	18.3*	77.5*	1.6*	8
C135	28*	18.8*	77.5*	1.6*	8
C137	17.5	17.8*	87.5*	1.6*	6
C138	18	12.5	62.5	1.5*	6
C139	13.5	13.8	62.5	1.3*	6
C140	11.5	14.3	60	1.4*	6
C102A	26.5	12.3	55	1.6*	10
C102B	43.5*	11.5	77.5*	1.6*	10
C102C	33*	9	60	1.5*	10
C103A	21	1.9	40	1.0	9
C103B	27.5	4.8	42.5	1.4*	9
C106B	25.5	1.6	35	1.0	9
C107A	17.5	1.8	25	1.0	9
C109A	39*	0.9	27.5	0.4	9
C44B	10	9.8	55	1.6*	7
CDD	6.5	7.3	30	1.0	UK
M.S.D.	15.7	8.5	24.8	.	.

*** indicates ratings not significantly different from highest rating within that column.**

Table 1.7: Traits for habitat value and competition with other species: observations for planting year 2007 ecotypes.

Ecotype	Average Density (1-5 scale)		Yield (Mg ha^{-1})		Height (cm)	
BOR #	2008	2009	Aug-08	Aug-09	Jun-08	Jul-09
C51	3.0*	3.0	5.9	5.8	26.0	26.2
C52	2.7*	3.2*	4.2	8.8	27.3	28.3
C53	2.8*	3.9*	4.8	10.2*	30.5*	51.2
C54	3.0*	4.3*	6.0	10.1	23.5	46.9*
C55	3.5*	4.4*	8.3*	14.4*	26.0	41.8
C60	2.7*	3.8*	8.7*	9.3	29.8*	57.9*
C61	3.0*	4.0*	7.4	10.8*	34.9*	48.5*
C62	3.0*	4.3*	6.8	12.4*	29.2*	50.7*
C63	3.0*	4.3*	7.9*	14.8*	29.2*	49.1*
C66	2.5	3.8*	4.0	11.1*	23.5	38.9
C67	3.3*	3.8*	7.1	13.7*	20.3	37.6
C68	3.3*	3.8*	8.3*	13.1*	22.9	32.9
C70	3.5*	3.6*	10.0*	14.2*	29.8*	38.6
C71	3.5*	4.3*	9.2*	9.4	27.9	53.7*
C75	4.0*	3.8*	11.4*	11.6*	21.6	32.1
C76	2.2	3.8*	4.3	10.0	22.9	39.6
C78	2.8*	4.5*	4.6	12.0*	29.8*	57.4*
C79	3.0*	3.5*	7.3	11.3*	27.9	41.0
C83	3.5*	4.0*	7.6*	13.3*	27.9	45.2
C85	2.8*	4.0*	7.3	12.8*	31.8*	50.8*
C87	3.0*	4.5*	6.1	10.0	24.1	49.7*
C90	2.2	3.5*	5.0	8.3	22.2	44.8
C92	3.0*	4.0*	5.5	8.0	27.9	44.7
C93	3.0*	4.0*	5.2	10.1	33.0*	55.0*
C94	2.7*	4.5*	5.0	9.3	35.6*	53.2*
C95	3.5*	3.8*	8.4*	10.9*	34.3*	50.3*
C96	2.8*	3.0	6.7	7.1	22.9	28.7
C98	2.8*	3.0	6.3	8.1	26.0	48.6*
C99	3.0*	3.3*	4.9	9.7	29.2*	44.2
C100	2.5	3.0	4.3	5.8	30.5*	38.3
C104	2.3	2.3	6.3	6.6	26.7	35.8
C111	3.0*	3.8*	5.7	12.9*	25.4	39.4
C112	2.2	4.0*	3.4	11.6*	26.7	30.5
C115	3.0*	4.4*	4.4	12.6*	22.9	27.9
C116	3.0*	4.3*	5.0	9.1	25.4	38.4
C117	3.8*	4.4*	6.7	10.5*	19.1	23.0
C118	3.3*	4.4*	4.8	11.0*	17.8	32.5
C120	3.0*	3.5*	8.7*	6.4	26.7	50.3*
C121	3.8*	3.8*	7.4	15.2*	24.8	38.2

C127	3.3*	3.9*	7.4	9.6	22.9	39.2
C129	2.8*	4.5*	5.3	11.8*	31.8*	55.0*
C130	3.2*	3.3*	8.7*	10.8*	32.4*	51.2*
C132	2.0	2.8	5.2	10.5*	33.0*	48.6*
C133	3.0*	4.4*	8.4*	13.5*	27.3	42.4
C134	2.7*	4.3*	3.9	13.4*	27.3	41.8
C135	3.3*	4.0*	7.6*	11.6*	33.0*	49.9*
C137	3.3*	4.2*	7.4	13.6*	26.0	41.8
C138	3.0*	3.5*	5.9	7.6	28.6*	51.2
C139	3.0*	3.3*	7.4	11.7*	25.4	53.2*
C140	3.0*	3.5*	5.9	11.1*	24.1	39.8
C102A	3.5*	2.0	11.3*	11.7*	19.7	40.4
C102B	3.3*	3.3*	8.6*	10.2*	24.1	37.1
C102C	3.3*	3.0	8.6*	9.7	21.0	33.0
C103A	2.3	2.5	5.3	5.0	26.0	58.4*
C103B	1.7	3.5*	5.0	10.9*	26.7	50.8*
C106B	2.3	3.0	4.2	9.4	21.6	44.5
C107A	3.0*	2.0	9.1*	8.0	20.3	39.4
C109A	2.5	2.0	6.1	7.0	21.6	32.5
C44B	3.0*	4.5*	5.3	12.0*	22.2	42.2
CDD	2.8*	3.3*	4.7	10.2*	19.7	30.2
Mean	**3.0**	**3.7**	**6.5**	**10.5**	**26.3**	**42.7**

*** indicates ratings not significantly different from highest rating within that column.**

Table 1.8: Planting year 2007 correlation of yield, height, density, and cover.

	2008 Pearson Correlation Coefficients			2009 Pearson Correlation Coefficients		
	Height	Density	Cover	Height	Density	Cover
Yield	-0.04	0.67*	0.12	0.06	0.47*	0.40*
Height		-0.13	0.24		0.05*	0.27*
Density			0.47*			0.51*

* indicates significance at p=.05

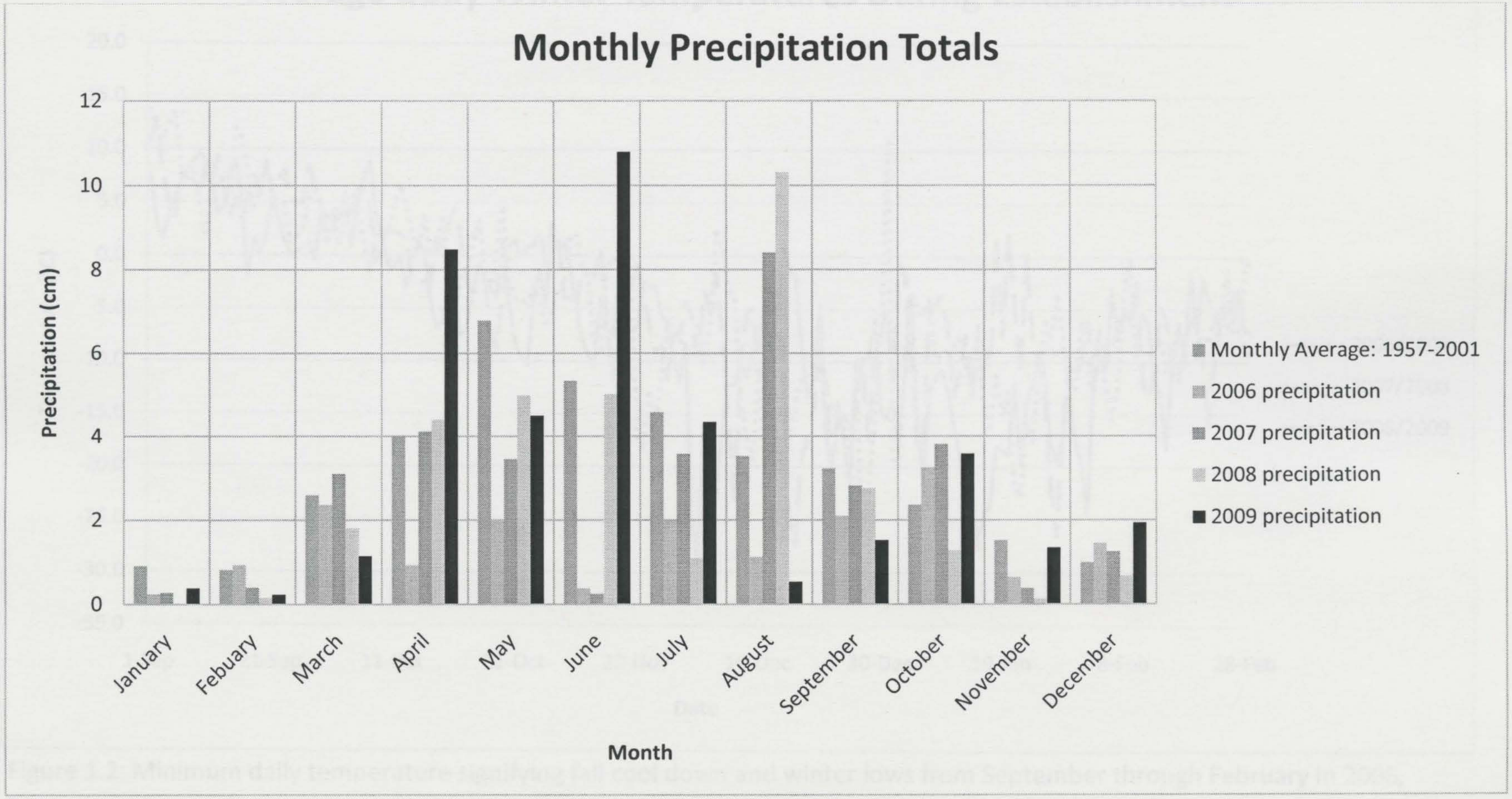

Figure 1.1: Precipitation by month from years 2006 to 2009 compared with historic average from 1957-2001.

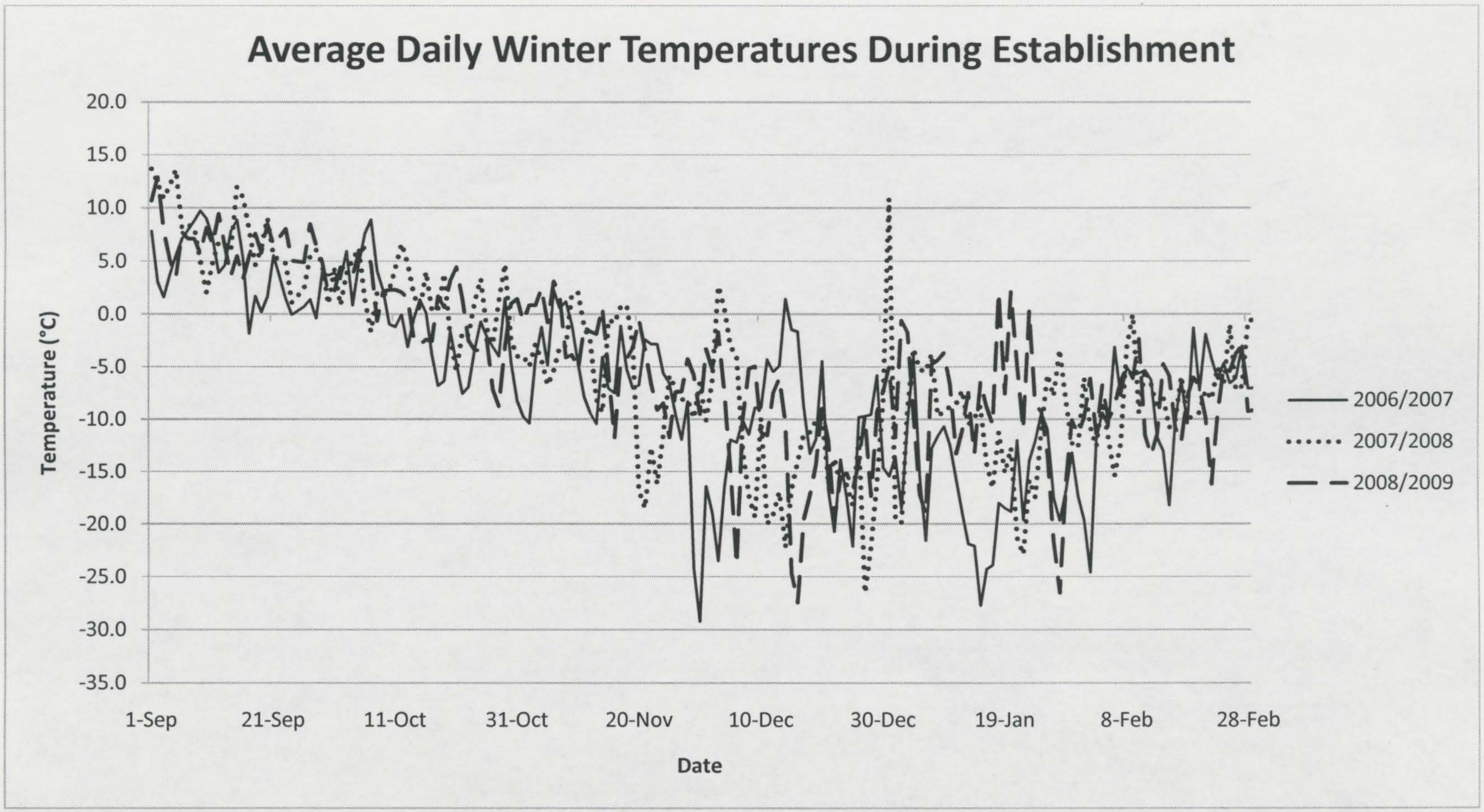

Figure 1.2: Minimum daily temperature signifying fall cool down and winter lows from September through February in 2006, 2007, and 2008.

Picture 1.1: Photo taken in 2008 of plot established with ecotype C135.


Picture 1.2: Photo taken in 2009 of plot established with ecotype C30.

Picture 1.3: Photo to display density of saltgrass ecotype C117 in field trials at CSU.


Picture 1.4: Photo taken in 2008 of plot established with ecotype C52.

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Chapter 2:

Saltgrass Germination Responses to Salinity with Different Seed Treatments

Abstract

Inland saltgrass (*Distichlis spicata* L. Greene) has great potential use as a revegetation species for riparian sites characterized by high salinity. In some revegetation situations, it may be most effective to use seeds rather than plugs or sprigs of saltgrass. Saltgrass has low germination rates due to seed dormancy issues. It has been shown in growth chamber studies that halophyte seed germination is increased with the use of the germination enhancing chemicals, Proxy and thiourea. In Experiment 1, as the average soil EC salinity increased from 3.5 to 7.6 dS m^{-1}, saltgrass seed germination was not affected. In Experiment 2, lower germination and plot coverage were observed in high salinity plots (soil salinity=12.4 dS m^{-1}) than in low salinity plots (soil salinity=4.0 dS m^{-1}). Our results indicate that Proxy solution at 5 mM a.i. enhanced saltgrass seed germination better than the other treatments at all salinity levels.

Introduction

Vigorous, pioneering native plant species that are tolerant of extreme environmental stresses early in establishment stages are an important component for re-establishment of a stable, diverse plant community on sites cleared of saltcedar (*Tamarix* spp.). Ecological restoration of sites impacted by saltcedar invasion and subsequent control of noxious species presents technical and conceptual challenges to the restoration of native species and desirable habitat. Because of the long duration of saltcedar occupation in dense, mature stands on many southwestern river systems, impaired surface and groundwater hydrology and high levels of soil salinity/alkalinity may be a significant constraint on revegetation success (Lair, 2006).

Saltgrass (*Distichlis spicata* L. Greene) can be found growing alongside saltcedar plants in the wild during early stage of saltcedar invasion. Saltgrass is a native species of grass with tolerance to many stresses. Revegetation efforts utilizing species which can be competitive with noxious weeds is one component required for successful reclamation efforts (Lair and Wynn, 2002).

Saltgrass is a perennial grass, which grows in a variety of environments, including sandy to heavy clay soils and can tolerate a wide range of pH levels (Qian et al., 2007). It is able to remain green in the heat of summer when all other grasses have gone dormant due to heat stress, and conversely can remain inundated for long periods of time because of advantages associated with having arenchyma (Bustan et al., 2005). Its main method of propagation in nature is via rhizome reproduction. Saltgrass typically thrives in ecosystems where soil characteristics limit other plant species. For example, some sites that are saline or sodic by nature are dominated by pure stands of saltgrass in Wyoming and Colorado (Linenburger et al., 2006; Bowman et al., 1985).

Saltgrass may be a good candidate to utilize on sites that are salt affected and have been determined to need revegetation. Direct seeding may be the most effective method to re-vegetate some sites due to lower material cost and ease of utilization with other species that may be planted in mixture with saltgrass. In general, seed germination undergoes three distinct phases. Phase one is characterized by rapid water imbibitions; in phase 2, considerable metabolic activity occurs while very little water is taken up by the seed; and phase 3 includes another rapid uptake of water coinciding with radicle growth and emergence (Taylor et al., 1998). However, seed germination of saltgrass may be significantly influenced by an endogenous biochemical inhibitor and a restrictive seed coat (Amen et al., 1970).

While saltgrass displays much salt tolerance at maturity, the seed germination processes appear more sensitive to higher EC levels. For example, in a study by Christensen and Qian (2004), saltgrass seed germination was significantly reduced as salinity levels reached 8 dS m^{-1}. In addition, a study conducted by Cluff and Roundy (1988) tested saltgrass seed to temperature and osmotic potentials. It was concluded that germination decreased with lower osmotic potential. Further, both percent seed germination and rate of germination decreased markedly when growth media water potential decreased from 0 MPa to -2 MPa (Cluff and Roundy, 1988).

Several efforts to improve saltgrass seed germination have been attempted. Qian et al. (2006) tested seed treatments including: hot water, hydrogen peroxide (H_2O_2), bleach, machine scarification, and stratification. In this experiment, scarification and stratification increased germination compared with control. In a growth chamber study, Shahba et al. (2008) tested germination under saline conditions with varied concentrations of different chemical treatments after seed had undergone machine scarification. Chemicals tested were: ethephon, fusicoccin, kinetin, thiourea, and Proxy (Bayer Environmental Science, Montvale, NJ). Proxy is used as a plant growth regulator with acive ingredient (a.i.) ethephon which promotes ethylene production in plants. Previous research suggests ethylene may stimulate seed germination, especially when seeds are exposed to salt and temperature stresses. Under saline conditions, 5 mM ethephon, 10 µM fusicoccin, 5 mM kinetin, 30 mM thiourea, and Proxy at 5 mM a.i. all increased germination percentage and the rate of saltgrass seed germination. Thiourea is a compatible osmoregulator. Thiourea could promote germination by acidification and softening of cell walls, or by activating the pentose phosphate pathway. However, Proxy treatment was the most effective.

Germination responses of halophytes to environmental conditions may determine their distribution in saline soils (Tobe et al., 2000). Ungar (1995) notes the germination of various halophyte seeds occurs at times when there is an optimal combination of day length, temperature, and salinity. In thesis work conducted by Judy Harrington (2000) at Colorado State University looking at overcoming seed dormancy in saltgrass, it was concluded that much variability exists amongst seed lots of saltgrass in a highly controlled environment. In field testing, a wide range of environmental variability influences test results. Therefore, the purpose of this experiment will be to scale-up the study conducted by Shahba et al. (2008) to determine saltgrass seed germination and establishment in the field. The goal is to determine saltgrass germination and establishment in the field at different soil salinity levels after scarified seeds are treated with Proxy and/or thiourea compared to a control of seed soaked in water to by-pass phase one of the germination process.

Methods and Materials

Two experiments have been conducted during summer of 2008 to observe seed germination and establishment of saltgrass after being treated with water (control), Proxy, and thiourea solution in field conditions under different soil salinity levels.

Experiment 1:

The first experiment was conducted from June 1 to July 31, 2008 at CSU Horticulture Research Center (HRC). In this experiment we looked at effects of four soil salinity levels and two Proxy treatments on germination and establishment of saltgrass. The seeds obtained for this study were an open pollinated, cycle 1 generation which was produced through breeding efforts at CSU. The salinity levels initially obtained were: salinity 1 (control): 2.5 dS m^{-1}; salinity 2: 4.5-6.5 dS m^{-1}; salinity 3: 7.5-9.5 dS m^{-1}; and salinity 4: 11.5-13.0 dS m^{-1}. Seed treatments (soaked for 48 hours in solution prior to sowing) included either 5 mM a.i. Proxy solution or control (no Proxy/water soaked seed). Experiment 1 was arranged in split plot design with salinity as the main plot factor and Proxy treatment as the subplot factor.

To obtain the salinity treatments, the top 2.5 cm of soil were amended with different rates of high salinity soils (~17.5 dS m^{-1}) obtained from north of Fort Collins, CO to reach desired salinity levels. Soils collected for this study were thoroughly mixed to uniformity and EC of the soil was determined in the lab by soil paste extraction method or using a soil salinity probe (Oakton Instruments EC Testr, Bozeman, MT). Native soil salinity levels at the HRC are in the range of 3.7-5.1 dS m^{-1} with pH of approximately 7.8. Because salinity levels dropped over time due to precipitation or irrigation water leaching events, plots were hand watered with high salinity water on 2, 4, 5, 7, 9, 11, and 14 days after seeding. For supplemental salinity irrigation treatments, 2 L of ocean salt solution with irrigation water salinity at 2, 5, 8, and 12 dS m^{-1} were uniformly applied to each plot.

All the seeds were cleaned and machine scarified as described by Qian et al. (2006). Seeds were sown by using a glass jar with a hole punched metal lid as a shaker to disperse seeds evenly over 1.49 m X 1.49 m plots. The seeds were sown at a rate of 150.7 kg/ha using milorganite as a dispersing agent at a rate of 247.6 kg N /ha. Plots were covered with white fabric material which was permeable to water and air, yet reflective of sunlight to reduce evaporation. Water was irrigated through an underground pop-up head irrigation sprinkler system at a rate of approximately 2 mm, twice a day: once in the morning and once in the evening. Plots were checked frequently to observe that seed beds remained moist.

Experiment 2:

The second experiment was conducted later in the summer (July 31 – September 19). Experiment 2 was arranged in a split plot design with salinity level as the main plot factor and seed chemical treatment as the subplot factor with four replications. Salinity treatments included low soil salinity level (3-4 dS m^{-1}) and high soil salinity level (12-18 dS m^{-1}). Seed chemical treatments included water soaked seed (control), Proxy at rates of 5 or 10 mM a.i. solution, and thiourea at a rate of 30 mM a.i. solution.

Individual subplots for this experiment were 0.5 m X 0.5 m. All the seeds were soaked in respective treatment solutions for 48 hours prior to sowing in the field.

Data Collection:

The data collection consisted of three parameters for both experiments. Because salinity is a dynamic variable in space and time, salinity measurements were taken frequently by soil paste extraction method or using the soil salinity probe. EC Testr-measured soil salinity was compared to conventional saturated paste extracted soil EC to assess data accuracy. Prior to seeding, plots with various salinity levels were tested for EC by EC Testr. The tested soil was collected for the conventional soil salinity measurement with saturated paste extract. EC Testr readings were linearly regressed against the conventionally measured salinity (Figure 1). Using the linear equation derived from the regression analysis, EC-Testr measured values were then adjusted to reflect salinity levels comparable to a conventionally measured salinity (saturated paste extraction).

Once seedling coleoptile emergence could be observed at the surface of the soil, seedling counts were taken multiple times on each plot with a ring measuring 6.5 cm in diameter which was randomly placed on the plots for each count. After 20 days following seeding for experiment 1 and 15 days after seeding for experiment 2, plots were rated for saltgrass coverage by visual ratings. Saltgrass coverage was estimated on a 0 to 100% scale by visually estimating percentage area covered with saltgrass in relation to the plot area.

Data Analysis:

Proc Mixed in SAS/STAT (version 9.2; SAS Institute, Cary, NC) was used to determine the effects of treatments and salinity levels on germination and saltgrass coverage over time. Least square means were estimated and graphed for germination count and saltgrass coverage.

Results

Experiment 1:

<u>Soil Salinity</u>

Soil salinity changed over time (Figure 2). Two days after seeding, precipitation amounting to 2.59 cm reduced soil salinity of all treatments except the control on Day 3. Therefore, following precipitation, waters with different salinity (~2, 5, 8, and 12 dS m^{-1}) were applied to different plots with salinity treatment 1, 2, 3, and 4, respectively. Typically, EC of salinity treatments 2, 3, and 4 were decreased significantly with every significant precipitation event that occurred. Precipitation lowered the salinity levels of the plots to roughly 2.5-4.5 dS m^{-1} within 24 hours (Figure 2).

Despite the substantially temporal difference, the rank of soil salinity among treatments remained the same throughout the experiment. The overtime average soil EC for salinity treatments 1 (control), 2, 3, and 4 were 3.5, 4.8, 5.9, and 7.6 dS m^{-1}, respectively.

Seed Germination

Emerged seedling germination counts commenced on the 11^{th} day after seeding. On 11 and 13 days after seeding, emerged seedling counts for either salinity level 2 or 3 were significantly higher than salinity level 4, suggesting that higher EC may delay germination of saltgrass seed (Figure 3). Although final cumulative seed counts were not different among salinity levels, at each salinity level, Proxy treated seed had significantly higher seedling counts than control treated seed (Table 1). Germination response is in agreement with Christensen and Qian (2004) who found that saltgrass seed germination percentage did not change until salinity reached 8 dS m^{-1}.

Saltgrass Establishment (Coverage)

Measurements of emerged seedling count transitioned into percent coverage when distinguishable levels were reached. Plot coverage data indicate that lower levels of soil salinity are preferred by saltgrass for growth across seeded surface area (Figure 4). Within 5 mM a.i. Proxy treatment, saltgrass at salinity level 1 had higher coverage than at salinity level 4 on all dates that observations were made, but not between salinity level 1 and salinity levels 2 and 3. Also, beginning on day 40 after seeding, saltgrass coverage at salinity level 2 was higher than that of salinity level 4 within 5 mM a.i. Proxy treatment. However, comparisons among salinity levels within no Proxy, control treated seed, indicated no difference.

At each salinity level, Proxy treatment significantly increased saltgrass coverage for all or most of the observation dates (Figure 5).

Experiment 2:

Soil Salinity

Despite the temporal difference, soil EC differed between high and low salinity treatments throughout the duration of this experiment (Figure 6). The average soil EC for the high and low salinity treatments was 4.0 and 12.4 dS m^{-1}, respectively.

Seed Germination

Seedling counts were compared among all treatments and between salinity levels. We were able to count emerged seedlings on the 7th day after seeding, suggesting that temperatures were more favorable for Experiment 2 than for Experiment 1. Seedling counts at the low salinity level for all treatments were always significantly higher than seedling counts at the high salinity level (Figure 7). Additionally, the results indicate that 5 mM a.i. Proxy solution had the greatest germination percent at both low and high salinity levels when compared to all other treatments (Figure 8).

Saltgrass Establishment (Coverage)

Percent plot cover ratings commenced on the 15th day after seeding for experiment 2. At low salinity level, both Proxy solution treatments had higher plot coverage than thiourea and control treatments (Figure 9). At high salinity level, 5 mM a.i. Proxy treated plots had greater coverage than thiourea and control treatments starting on the 28th day after seeding. Moreover, 5 mM a.i. Proxy treated plots had greater coverage than 10 mM a.i. Proxy treated plots starting 40 days after seeding.

Discussion

Results from Experiment 1 indicate that soil salinity below 7.6 dS/m did not significantly influence germination response. Several studies show that the effect of salinity on germination of different species and even genotypes of species varies considerably with temperature and salinity (Badger and Ungar, 1989; Morgan and Myers, 1989; Myers and Morgan, 1989). Moreover, the response of halophytic seeds to alternating temperatures and soil salinity levels is of ecological significance (Gulzar and Khan, 2000). Some studies indicate that seeds of halophytes can remain viable for an extended period of exposure to salt stress and germinate when conditions are favorable (Khan and Ungar, 1997; Li et al., 2010; Zia and Khan, 2004). Ungar (1987) notes that halophytes establish from seeds in saline habitat during periods following high precipitation events, low evaporation, and thus, lower EC levels. Because the seeds were soaked in treatment solutions for both experiments, and in other words, the need for phase one of the germination process to occur in the field was by-passed, we were primarily observing the effects of ions on the metabolic phase of germination, which is phase 2 and phase 3 thereafter of germination. During the start of Experiment 1, a precipitation event that occurred may have allowed mitigation of salt effects for either phase 2 or 3 on germination of the seed (Figure 2), thus allowing for no differences in germination counts across salinity levels. However, the decreased germination rate of Experiment 2 is believed to be a direct result of salt ions causing inhibition of seedling emergence. The soil salinity reached a higher level in Experimental 2 than Experimental 1.

Proxy at 5 mM significantly enhanced germination of saltgrass seed in both experiments. It is unknown exactly why Proxy at 5 mM a.i. solution enhances saltgrass seed germination. The active ingredient in Proxy is ethephon, a chemical which increases production of ethylene in plants. The other ingredients that make up Proxy are proprietary. In plant metabolism, ethylene is considered to be a powerful natural regulating hormone that acts and interacts with other recognized plant hormones in trace amounts (Cho et al., 1988). Shahaba et al. (2008) found that 5 mM Proxy was more effective than ethephon as a treating agent for increasing saltgrass seed germination. In experiment 2, at low salinity level, 5 mM a.i. Proxy solution increased emerged seedling count by 33% while 10 mM Proxy solution had 9% increase, and thiourea increased germination by 2% in comparison with control seedling counts. In contrast, at high salinity level, 5 mM a.i. Proxy solution increased emerged seedling count by 183% while 10 mM Proxy solution enhanced germination by 68%, and thiourea increased emerged seedling count by 19%.

In seed germination, ethylene is produced in the embryonic axis to reduce cell expansion allowing for more cell divisions which increases girth of the embryonic stem and hence capabilities of protrusion from the seed coat (Ashraf and Foolad, 2005). Scarification weakens the seed coat in addition to allowing for easier water uptake to occur, therefore, ethylene production may further enhance ability of embryo to protrude the seed coat. In comparison, it is plausible to think that higher concentrations of Proxy (10 vs. 5 mM a.i.) may create an imbalance of plant hormones or react negatively so as to inhibit some seed from germinating.

Plot coverage of saltgrass appeared inhibited as salinity increased in soil. Even in Experiment 1 where seed germination was similar at all salinity levels, high salinity treatment decreased saltgrass coverage in this study due to a reduced ability to grow. Unfortunately, we only measured soil salinity up to 15 days after seeding. It is possible that soil salinity increased thereafter. While mature plants of saltgrass are very tolerant of salt, seedlings may not have fully developed mechanisms of dealing with higher salinity effectively. Previously, Alshammary et al. (2004) found that shoot growth of mature saltgrass stand did not decline significantly when salinity increased from 2 to 23 dS m^{-1}.

Seeding Date

Seed emergence occurred faster in Experiment 2 than Experiment 1, which may have been a result of more ideal temperatures (Table 2 and Table 3). It was shown that optimum warm period temperatures for saltgrass seed germination were above 30° C but less than 60° C and that no saltgrass seed germinated at less than 20° C difference between cold and warm period temperatures (Cluff and Roundy, 1988). In both of our experiments, while atmospheric temperatures were recorded, seedbed micro-climate temperatures were not.

Shahba and Qian (2008) determined that earlier seeding date of May or June provides adequate plot cover (defined as >80%) by September. This study confirmed those finding as in experiment 1 at all salinity levels and both seed treatments did provide adequate cover by September. However, while emerged coleoptiles appeared earlier in Experiment 2 compared with Experiment 1, no treatment provided adequate cover of plots, even at the low salinity level (data not shown).

Conclusions

In Experiment 1, when salinity levels were at or below 7.6 dS m^{-1}, saltgrass seed germination was not affected. However, in Experiment 2, salinity at 12.4 dS m^{-1} inhibited saltgrass germination and establishment. In both Experiments, 1 and 2, Proxy at 5 mM a.i. solution significantly enhanced seed germination of saltgrass at increased salinity levels when compared to all other treatments. In Experiment 1, it was determined that when saltgrass seed is planted in early June, percent cover of all plots were able to reach adequate levels by the end of the growing season. However, for saltgrass to be planted at the end of July in Colorado is not sufficient time to allow for saltgrass to adequately cover seeded ground space.

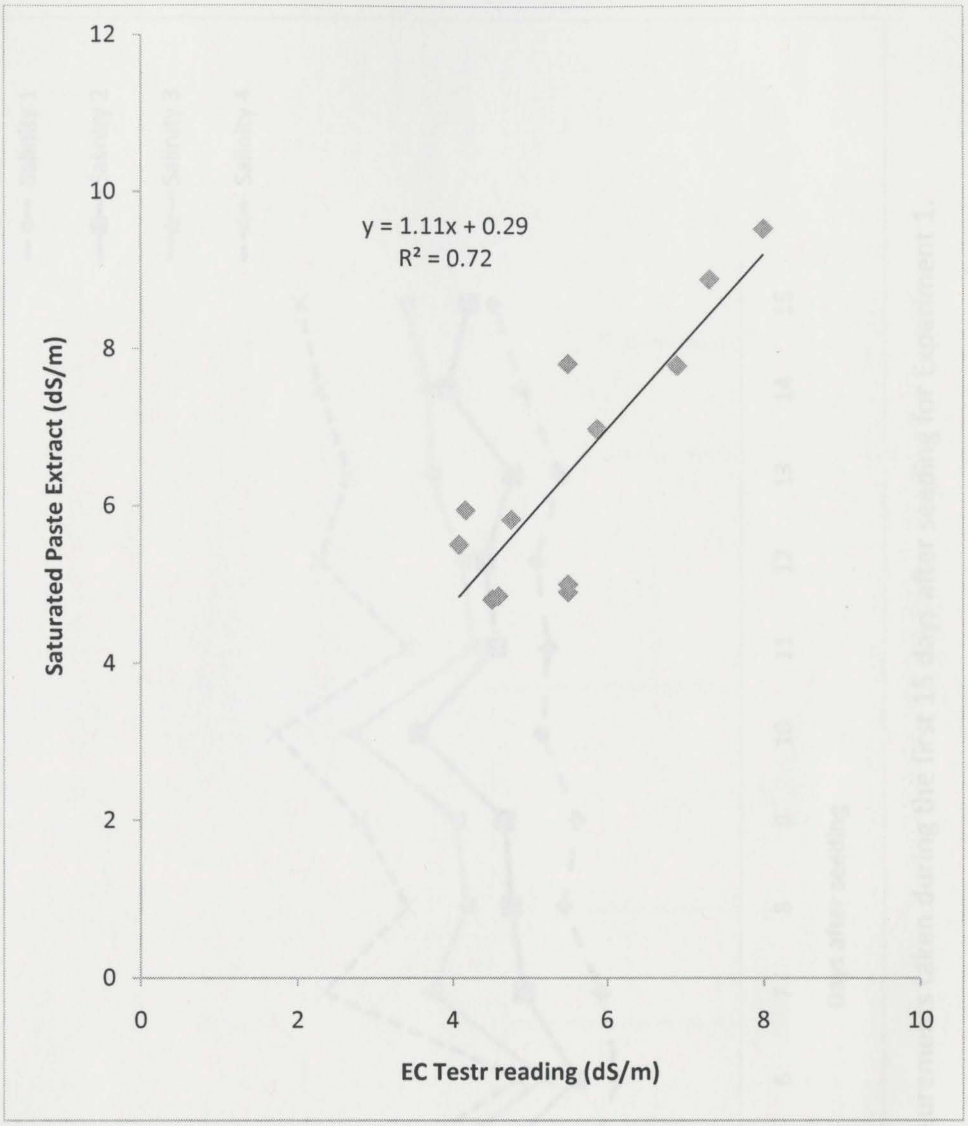

Figure 2.1: Linear regression of EC Testr measured soil electrical conductivity (EC) and conventional saturated paste extract EC.

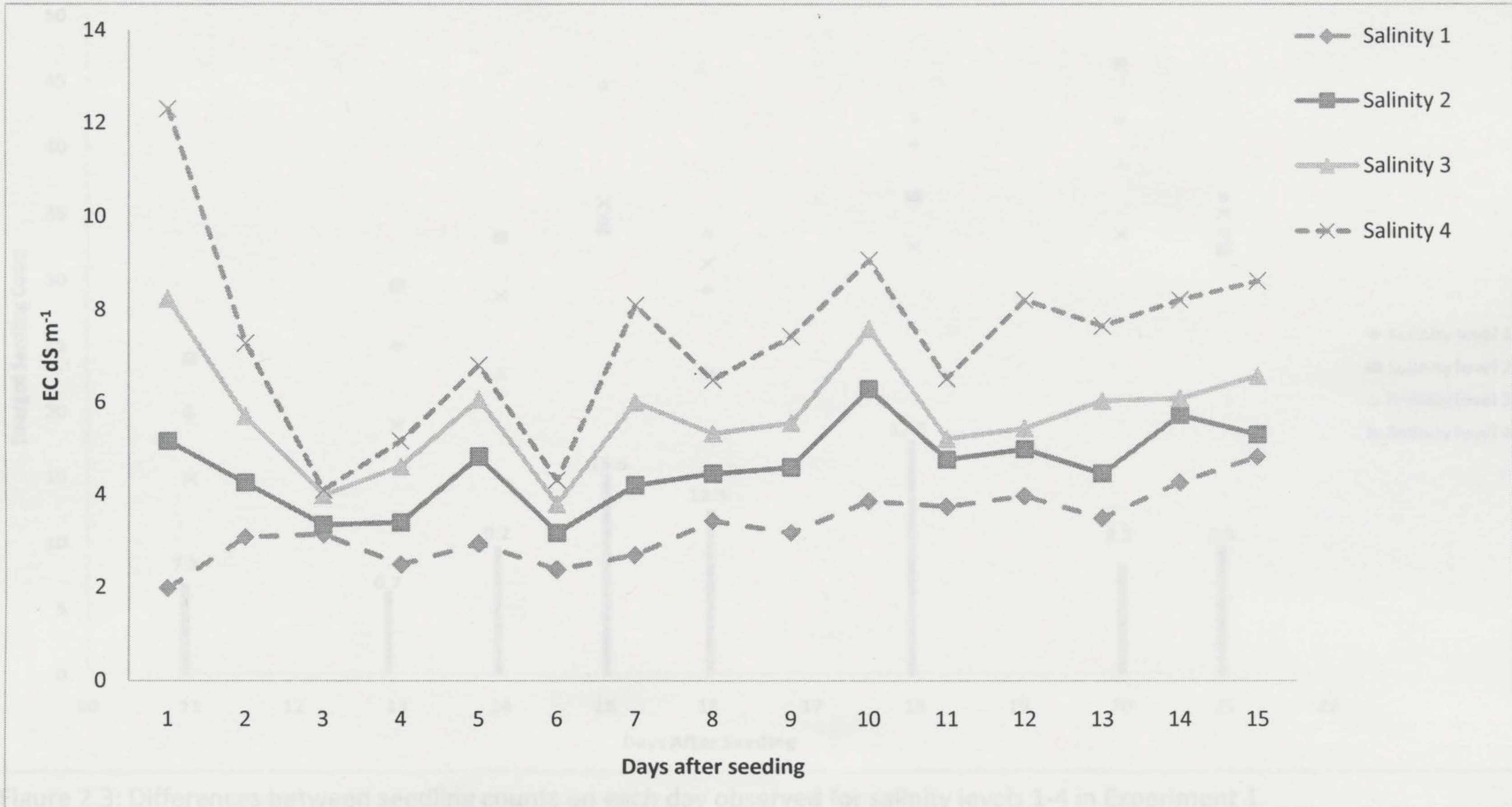

Figure 2.2: EC Testr probe soil salinity measurements taken during the first 15 days after seeding for Experiment 1.

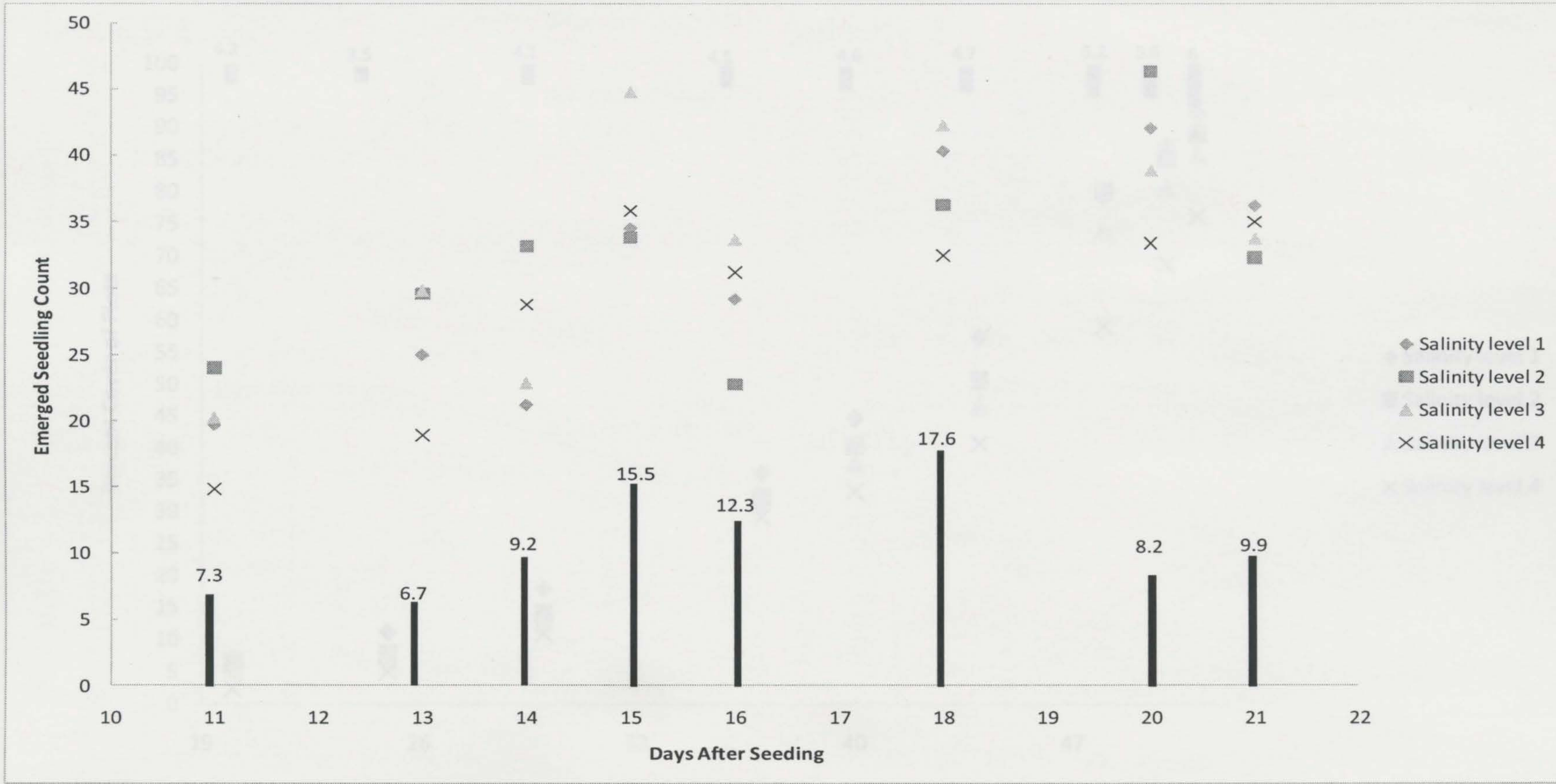

Figure 2.3: Differences between seedling counts on each day observed for salinity levels 1-4 in Experiment 1.
*Black bars on X axis indicate least significant difference on the day after seeding in which observations were made.

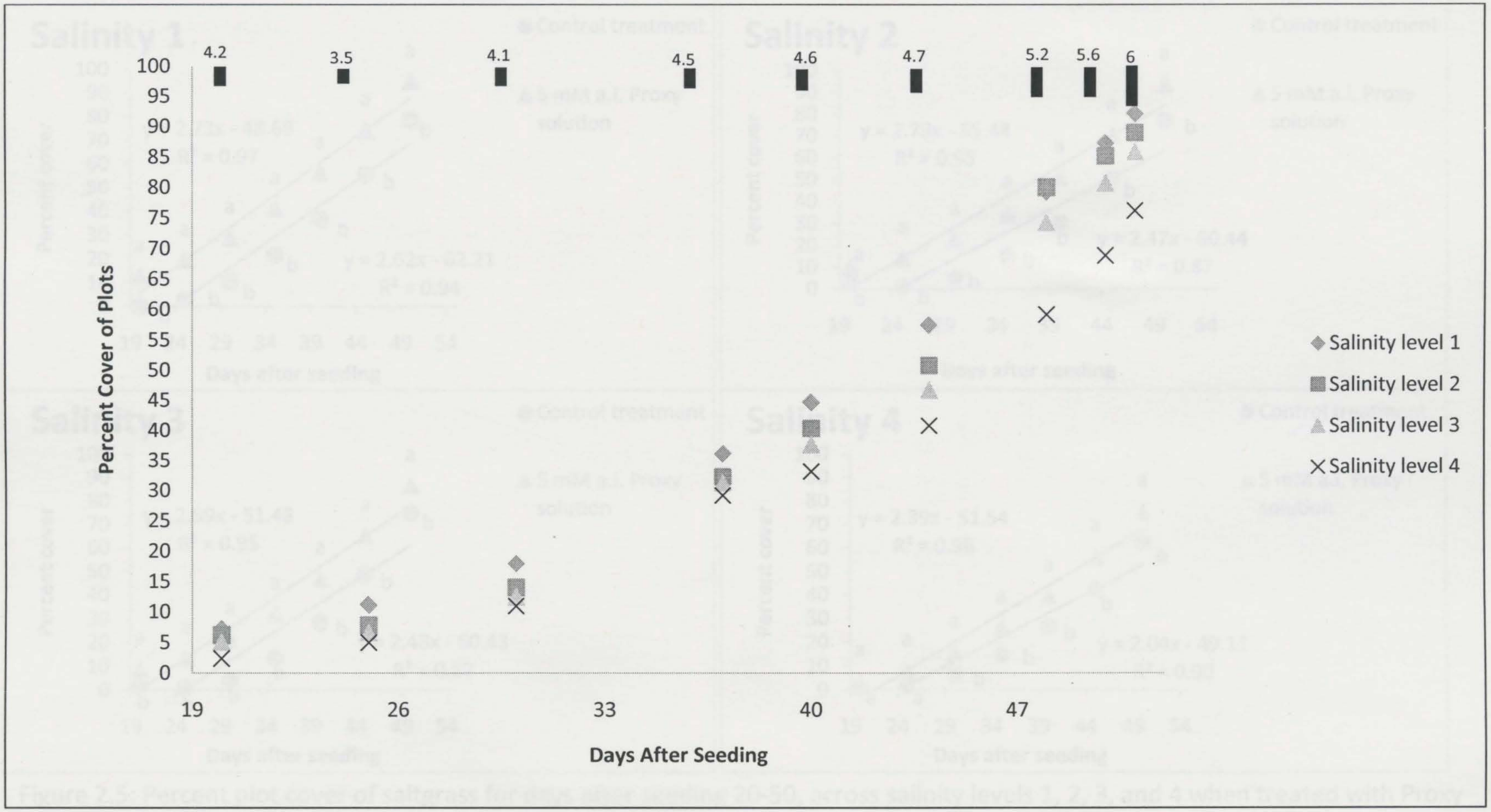

Figure 2.4: Percent cover of plots at all salinity levels in Experiment 1.

*Black bars above day after seeding observations indicate least significant difference.

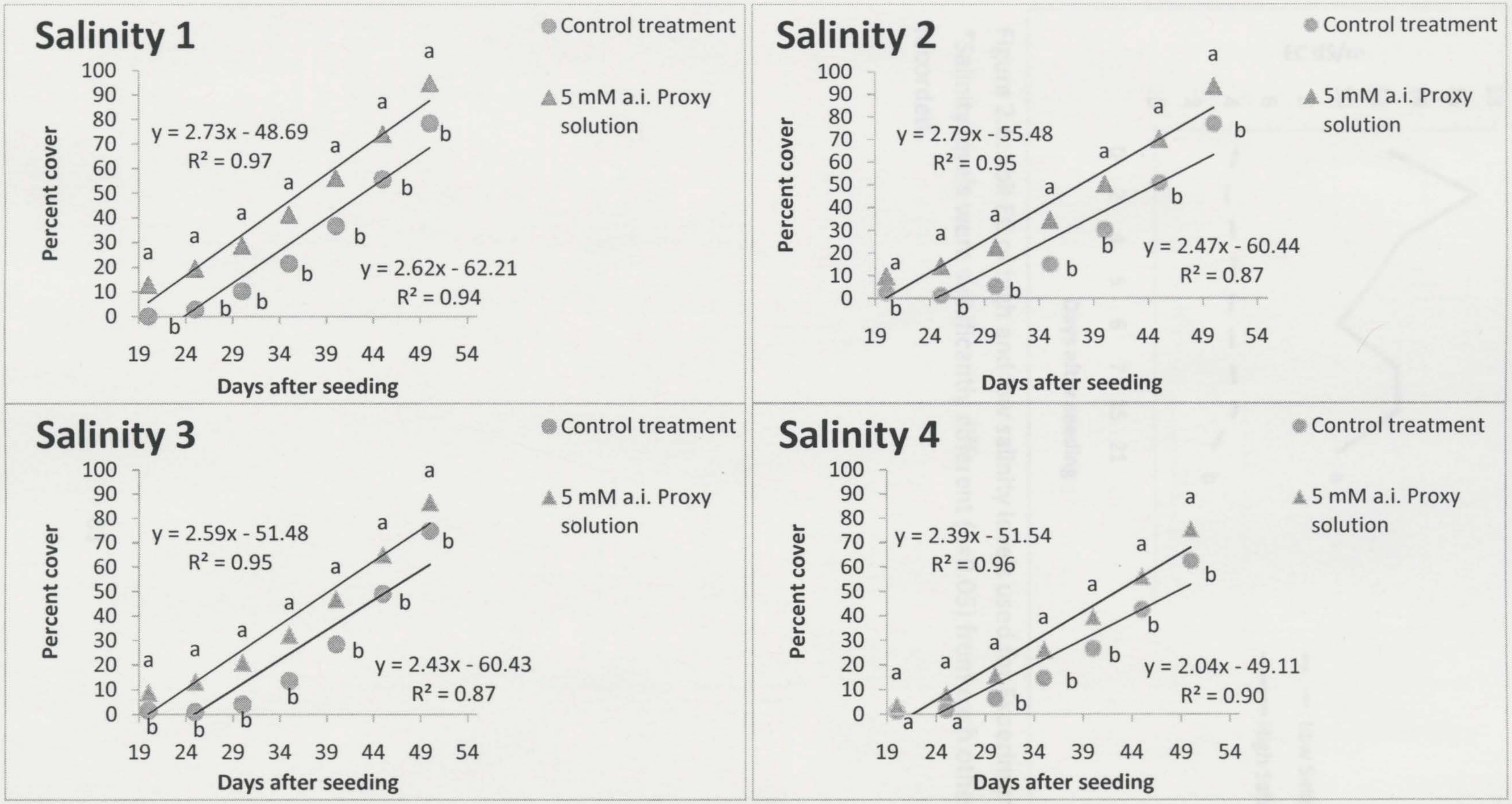

Figure 2.5: Percent plot cover of saltgrass for days after seeding 20-50, across salinity levels 1, 2, 3, and 4 when treated with Proxy at 5 mM a.i. compared with control in Experiment 1.

*Observation points for days after seeding with different letters are significantly different at p=0.05 for control and Proxy data.

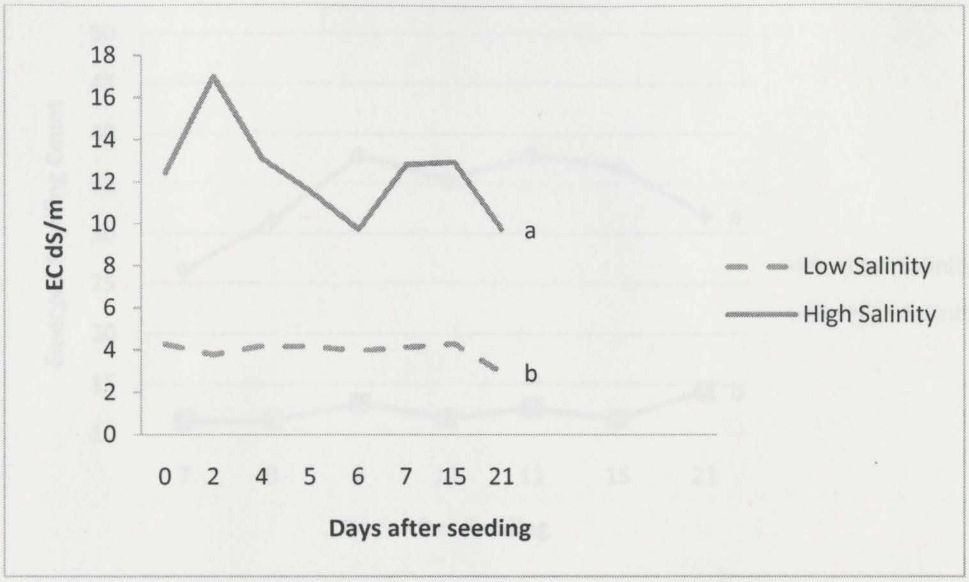

Figure 2.6: Soil EC of high and low salinity levels used for Experiment 2.
*Salinity levels were significantly different (p=0.05) from each other on all dates recorded.

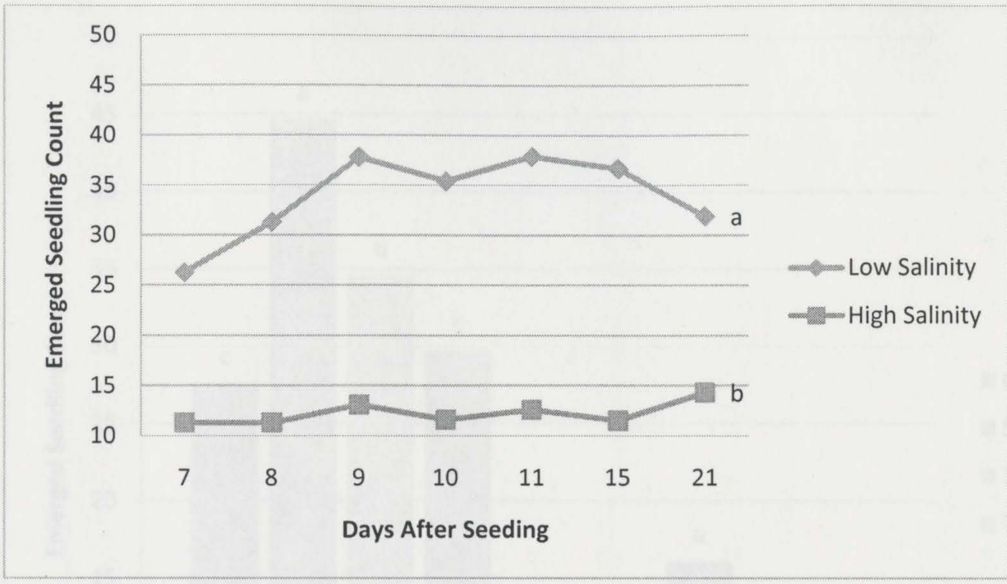

Figure 2.7: Emerged seedling count at high and low salinity levels in Experiment 2.
*Counts were significantly different (p=0.05) from each other on all days measured.

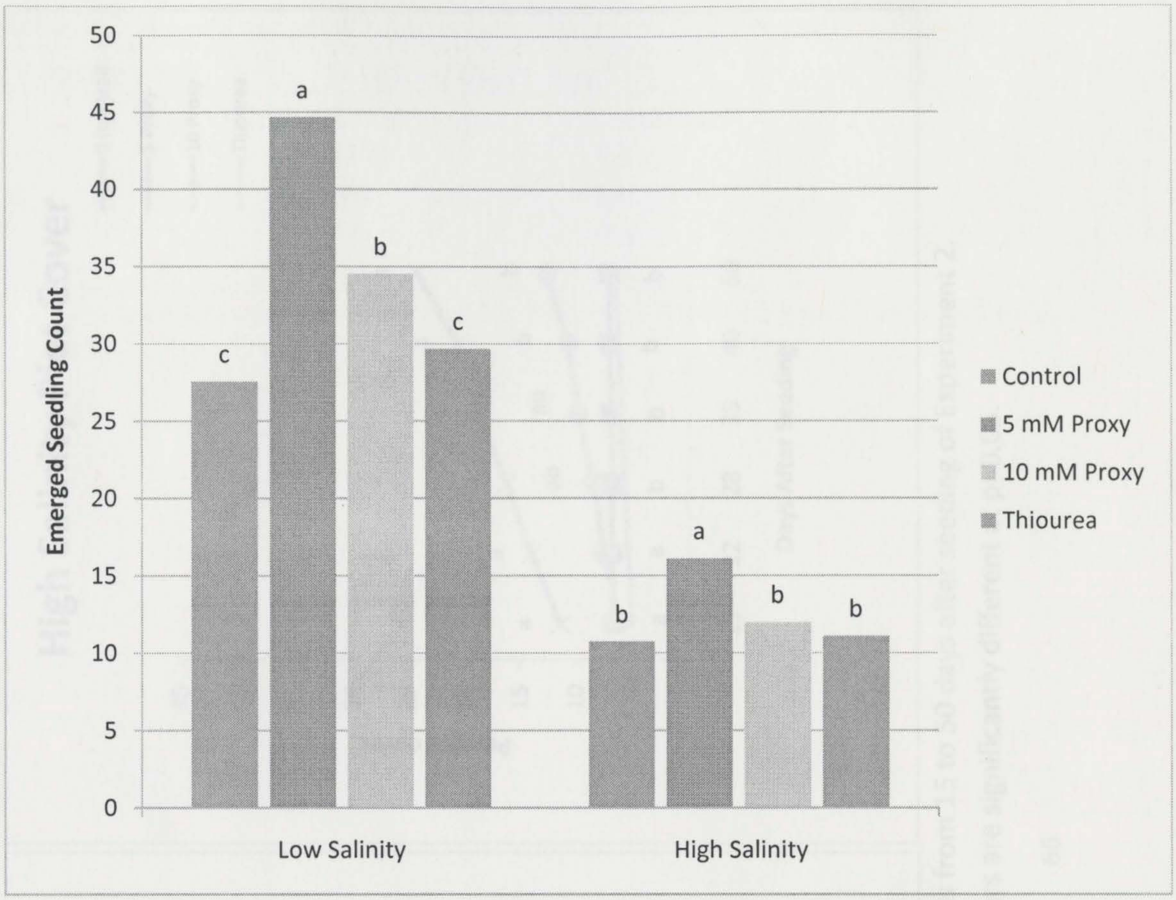

Figure 2.8: Number of saltgrass seedlings emerged from 7 to 15 days after seeding in Experiment 2.

*Bars with different letters are significantly different (p=0.05) among chemical treatment at high and low salinity levels, respectively.

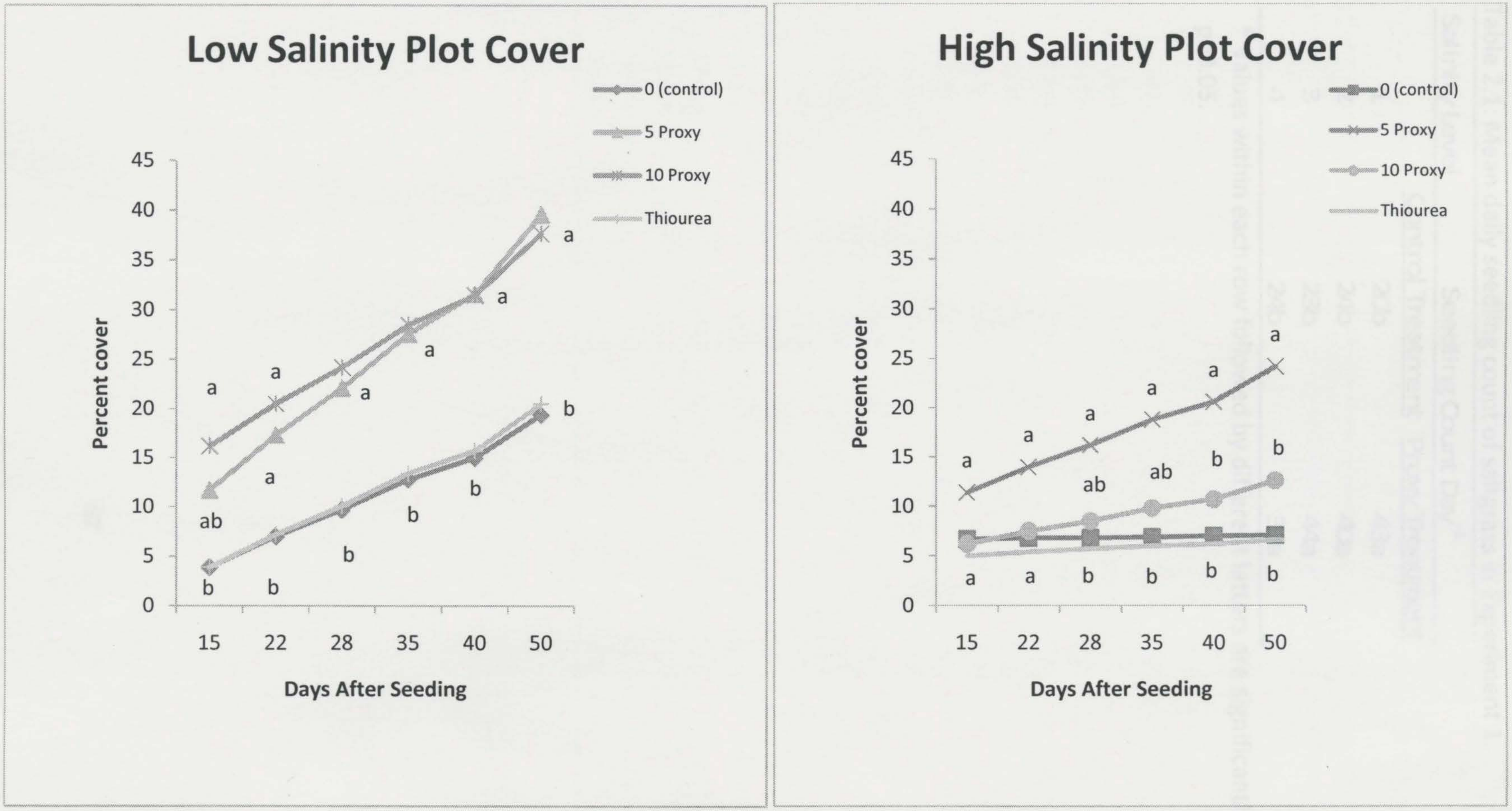

Figure 2.9: Percent cover of plots at low and high salinity levels from 15 to 50 days after seeding of Experiment 2.
*Observation points for days after seeding with different letters are significantly different at p=0.05.

Table 2.1: Mean daily seedling count of saltgrass in Experiment 1.

Salinity Level	Seedling Count Day^{-1}	
	Control Treatment	Proxy Treatment
1	20b	43a
2	24b	40a
3	23b	44a
4	24b	36a

* Values within each row followed by different letters are significantly different at p=0.05.

Table 2.2: Atmospheric temperature during seed germination for Experiment 1.

Date	High Temperature	Low Temperature	Difference
1-Jun	27.5	10.7	16.7
2-Jun	28.8	8.8	20.0
3-Jun	26.2	10.7	15.5
4-Jun	19	10.5	8.5
5-Jun	15.7	7.6	8.0
6-Jun	24.9	8.7	16.1
7-Jun	20.1	6.3	13.7
8-Jun	20.5	7.6	12.9
9-Jun	22.6	6.3	16.2
10-Jun	30.5	5.8	24.6
11-Jun	20.4	8	12.4
12-Jun	21.8	2.7	19.1
13-Jun	24.3	4.5	19.7
14-Jun	29.4	6.8	22.5
15-Jun	27.1	11	16.1
16-Jun	23.4	13.7	9.6
17-Jun	32.0	10.5	21.5
18-Jun	29.7	10.1	19.6
19-Jun	27.0	11.9	15.1
20-Jun	27.2	11.0	16.1
21-Jun	30.1	8.6	21.5

Table 2.3: Atmospheric temperature during seed germination for Experiment 2.

Date	High Temperature	Low Temperature	Difference
31-Jul	32.4	13.4	19.0
1-Aug	36.7	13.7	23.0
2-Aug	36.2	13	23.2
3-Aug	34.6	13.7	20.8
4-Aug	33	11.6	21.3
5-Aug	29.2	13	16.2
6-Aug	30.6	13.4	17.2
7-Aug	25.3	15.9	9.4
8-Aug	26.0	15.8	10.1
9-Aug	31.5	14	17.5
10-Aug	26.8	15.8	11
11-Aug	29.6	10.6	18.9
12-Aug	30.3	10.0	20.2
13-Aug	30.8	8.5	22.3
14-Aug	25.1	10.5	14.6

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