Screening of Alfalfa (*Medicago sativa*) Cultivars for Salt Tolerance in West Texas

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ABSTRACT

Alfalfa (*Medicago sativa*) is a very important crop commodity in the Trans-Pecos region of Texas and New Mexico where salinity problems occur in the soil and irrigation water resources. There has been significant research in the area of salt tolerance of alfalfa but there is need for screening current and experimental alfalfa cultivars to assist growers with variety selection. Due to the complexity of salinity tolerance in plants, it is also necessary to compare laboratory and greenhouse screening methodologies and results to field conditions. These experiments were designed to evaluate commercial and experimental alfalfa cultivars at different growth stages and varying salinity concentrations and assess correlations between laboratory, greenhouse and field experiments. In addition to salinity tolerance, there is a need to evaluate alfalfa cultivars for potential quality differences between varying fall dormancy (FD) ratings to assist growers in cultivar selection.

The first experiment evaluated plant germination under increasing levels of salt concentrations conducted in the laboratory. Statistical differences were observed for percentage germination among cultivars which could influence stand establishment of alfalfa in saline conditions. These differences in turn are exhibited in the IC(50) values which reflect osmotic potential required to inhibit 50% of seed from germination. The second experiment evaluated forage production under two levels of salt concentration conducted in the greenhouse. There were no significant differences in raw or transformed SCR values among the 32 cultivars at Cut 2, Cut 3 or Cut 4 when subject to analysis of variance. SCR values were then evaluated for statistical differences using the three harvests as replications over time. Significant differences in raw and transformed

SCR values were found between cultivars with Cut 2, Cut 3 and Cut 4 serving as replications over time. The third experiment evaluated forage production under saline field conditions that exist at the Texas AgriLife Research Station west of Pecos, TX. Throughout the two-year project, significant differences among cultivar yields were observed only 4 out of the 13 (31%) harvests at alpha = 0.05 (6 out 13 exhibited significant differences when using alpha = 0.1). There were no significant differences when two-year totals or when two-year averages were subjected to ANOVA. It should be noted that all 12 of the cultivars in this experiment were selected for the potential to tolerate saline conditions (based upon breeding or adaptation of the cultivar) and therefore all were expected to perform well under saline conditions. However, the differences seemed to become more apparent later in the growing season, perhaps indicating salt stress has a more severe impact over the long term (i.e., salt loading during the year, salt loading during the life of the stand, etc).

When comparing data from the three experiments several principles become evident. First, as percentage germination increases regrowth potential decreases indicating a negative correlation. However, as percent germination increases, potential yield under saline conditions increases. Second, as percent germination increases the potential field production and yield also increases. Third, as Salt/Control Ratio (SCR) values increase, production potential in the field decreases indicating a negative correlation. However, as average saline yields in the greenhouse increase, potential yield under saline conditions in the field also increased.

The fourth experiment compared fall dormancy (FD) ratings and forage quality data and no significant differences between FD8 and FD10 alfalfa cultivars evaluated were found to exist over the course of a growing season. The data does, however, show that differences can exist at individual cuttings during the growing season although it seems to be more the exception than the rule. Therefore, forage quality differences between FD8 and FD10 alfalfa cultivars may occur, but in general should not be a factor for producers when selecting cultivars.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADF – Acid Detergent Fiber

ANOVA – Analysis of Variance

CEC – Cation Exchange Capacity

CP – Crude Protein

DAP – Days After Planting

dS/m – decSiemens per meter

EC – Electrical Conductivity

ESP – Exchangeable Sodium Percentage

FD – Fall Dormancy

FIELD – Used to denote the field production experiment

GERM – Used to denote the laboratory germination experiment

GRIN – Germplasm Resource Information Network

IC(50) – Osmotic pressure required to inhibit germination of 50% of seed

LSD (0.05) – Least Significant Difference at the 5% confidence level

mM – millimoles per liter

mmhos/cm – millimhos per centimeter

mPa – megapascals

NASS – National Agricultural Statistics Service

NDF – Neutral Detergent Fiber

NPGS – National Plant Germplasm System

NS – No Significant Differences

ppm – parts per million

PROD – Used to denote the greenhouse production experiment

RDP – Rumen Degraded Protein

RFQ – Relative Feed Quality

SAR – Sodium Absorption Ratio

SCR – Salt/Control Ratio

TDN – Total Digestible Nutrients

TDS – Total Dissolved Salts

USDA – United States Department of Agriculture

CHAPTER I
INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a very important crop commodity in the Trans-Pecos region of Texas and New Mexico. This region is limited in crop production due to several factors, including: low rainfall, saline water (surface and ground), saline soils and high evaporation rates. Salinity levels in irrigation water in far west Texas have been observed up to 6,000 ppm dissolved salts (Miyamoto et al., 1984). Recent analysis of salinity levels in the middle Pecos River averaged 3,500 and 6,150 mg L^{-1} at Malaga and the Red Bluff Reservoir release and upwards of 12,000 mg L^{-1} at Girvin (Miyamoto et al., 2008). The Texas AgriLife Research Station in Pecos, TX, the location where the research project was conducted, has well water that ranges from 2,700 ppm to 3,853 ppm total dissolved salts (TDS). Management practices in the saline region of far west Texas have changed over time, especially around the Pecos area, as some producers have changed from pivot irrigation on alfalfa fields to flood irrigation to minimize salt buildup (Trostle, 2005).

Alfalfa is a very important forage crop (especially to the feed and livestock industry) and in 2002 an estimated 2,516 farms in Texas produced 164,069 acres of alfalfa hay (NASS, 2002). In west Texas it is estimated that 358 farms in the 23 county Trans-Pecos region produced 29,335 acres of alfalfa hay (only 358 of the 400 farms actually reported acreage) and 238 farms in the 44 county High-Plains region produced 28,793 acres of alfalfa hay (only 238 of the 461 farms actually reported acreage) (NASS, 2002) (Table 1.1 and Table 1.2).

Alfalfa has been characterized as a moderately salt sensitive plant (Maas, 1987) but tends to grow well in the region and is grown on a relatively wide scale. There has been significant research in the area of salt tolerance of plants (specifically alfalfa), but there is a need for the screening of current and experimental alfalfa cultivars to assist growers with variety selection as well as to compare laboratory and greenhouse screening methodologies and results to field conditions. Additionally, data generated from field trials of alfalfa cultivars is relied on heavily by breeders, seed dealers and producers (Casler and Undersander, 2000).

The objective of this research was to evaluate the salt tolerance of commercial and experimental cultivars of alfalfa using three criteria: salt tolerance at germination, forage production under salt stress and production under saline field conditions. These experiments evaluated these criteria under laboratory, greenhouse and field experiments, respectively. A total of thirty-two varieties were analyzed in the two initial stages of research. Twelve cultivars were selected to be included in the field trial conducted over the period of two years based upon salt tolerance exhibited in the first two experiments and/or relevance to farmers in the area. All experiments were conducted at the Texas AgriLife Research Station in Pecos, Texas.

Table 1.1: Alfalfa farms and acres reported by USDA in 2002 for a 23 county area located in the Trans-Pecos region of Texas

County	Farms	Acres
El Paso	222	4,810
Hudspeth	41	15,437
Martin	17	940
Midland	16	848
Pecos	18	4,341
Presidio	22	720
Reeves	19	2,225
Ward	3	14
TOTAL	**358**	**29,335**

The following counties reported a cumulative total of 42 farms growing alfalfa but did not provide acreage totals for the 2002 report: Andrews, Brewster, Crane, Crockett, Culberson, Ector, Glasscock, Howard, Jeff Davis, Loving, Reagan, Terrell, Upton, Val Verde and Winkler.

Table 1.2: Alfalfa farms and acres reported by USDA in 2002 for a 42 county area in the High-Plains region of Texas

County	Farms	Acres
Bailey	19	2,832
Carson	3	1,137
Cochran	7	723
Collingsworth	31	1,883
Dawson	18	1,965
Deaf Smith	10	569
Dickens	13	2,124
Donley	24	2,076
Gray	11	811
Hall	4	121
Hansford	7	754
Hartley	18	4,113
Hemphill	4	386
Lamb	21	4,925
Lipscomb	7	2,494
Lynn	10	399
Moore	3	194
Swisher	12	581
Wheeler	16	706
TOTAL	**237**	**28,793**

The following counties reported a cumulative total of 224 farms growing alfalfa but did not provide acreage totals for the 2002 report: Armstrong, Borden, Briscoe, Castro, Crosby, Dallam, Floyd, Gaines, Garza, Hale, Hockley, Hutchinson, Lubbock, Motley, Ochiltree, Oldham, Parmer, Potter, Randall, Roberts, Sherman, Terry, Yoakum.

CHAPTER II
REVIEW OF LITERATURE

Introduction to Alfalfa

Alfalfa (*Medicago sativa* L.) is a perennial, warm season legume that is widely grown in the United States of America and Canada. Alfalfa is often referred to as "Queen of the Forages" for its ability to produce forage that is high in crude protein and total digestible nutrients (Barnes et al., 1988). Another name commonly used for alfalfa is lucerne. Plant longevity of alfalfa can reach up to 30 years in length and alfalfa requires deep soils and near neutral pH for optimum production (Stichler, 1997). The ability of alfalfa to fixate atmospheric nitrogen makes it ideal for crop rotation systems. Proper inoculation of alfalfa seed with *Rhizobium* bacteria will ensure nodulation and nitrogen fixation by alfalfa plants (Hall, 1997).

Establishment of alfalfa is critical in long term stand life and production. Alfalfa seed is extremely small (~225,000 seeds per pound) and it is recommended that seed be planted on the soil surface or no deeper than ½ to ¾ inches deep in a well prepared soil bed (Oklahoma State University, 2000). Planting is recommended for the fall of the year due to weed, insect and water issues that arise with spring plantings (Trostle, 2003). The time frame for this fall planting is recommended from August 20 to October 1. Because of alfalfa's high production level it has an unusually high plant nutrient requirement and proper fertility management at the pre-plant stage and of mature stands is essential to long term production (Stichler, 1997).

Plants will generally bloom from 28 to 30 days after each cutting at a height ranging from 1.5 feet to 2 feet. Harvesting at first flower has resulted in optimized quality, yield and stand persistence (Sheaffer et al., 1988). Alfalfa has a high water requirement and approximately 10 inches of water are needed to produce a ton of forage (Stichler, 1997). Alfalfa does have good drought tolerance via its ability to go dormant during extended dry conditions and then to recover once adequate moisture becomes available (McWilliams, 2002). Experiments evaluating the use of controlled deficit irrigation of alfalfa indicate the potential annual water savings of 600 to 850 mm (Sacramento Valley) and 280 to 530 mm (Klamath Valley). This strategy may alleviate conflicts between agricultural and municipal water use demands with some yield reduction but no long-term impacts on alfalfa stands (Putnam et al., 2005).

Introduction to Salinity

Salinity is a problem that affects many arid and semi-arid regions of the world and generally is a major concern in areas of irrigated agriculture (Clark et al., 2000). Salinity is defined as the total dissolved concentration of major inorganic ions (i.e., Na, Ca, Mg, K, HCO_3, SO_4 and Cl) in irrigation, drainage and groundwaters (Rhoades et al., 1992). About one-fifth of irrigated agriculture is adversely affected by soil salinity which necessitates the development of salt-tolerant crop production to ensure sustainable food production (Chinnusamy et al., 2005). Conversely, salinity is a very complex issue to characterize in terms of the factors that cause it and the parameters for plant tolerance. This complexity is also due to the many factors that influence a plants response to salinity, including: plant, soil, water, environmental and cultural (Maas, 1987). The interactions of these various factors may be too complicated to make field experiments valid (McKimmie and Dobrenz, 1987). Soil salinity has been characterized as one of the most variable properties of soils (Miyamoto, 1988). Additionally, salinity can have different affects on a plant depending on the stage of development of the plant (Bernstein and Hayward, 1957). Different aged alfalfa seed lots from the same germplasm source were evaluated for tolerance at germination and significant differences were observed when expressed as a proportion of the nonsaline germination of each lot (Smith and Dobrenz, 1987b).

Salt tolerance evaluation in greenhouse environments may not correlate with field evaluations (Cluff, 1997). The tolerance of plants to salinity is typically evaluated in three ways: 1) the ability of a plant to survive on saline soils, 2) the absolute plant growth or yield, and 3) the relative growth or yield on saline soil compared with that on non-saline soil (Maas, 1987). The ability to simply survive under saline conditions does not necessarily correlate to higher yields under salt stress (Smith and Dobrenz, 1987

<u>Characterizing Salinity</u>

In order to gain a comprehensive understanding of salinity problems it is vital to understand how to characterize salinity levels, sodium levels and the different types of salt affected soils. Generally, salinity levels are quantified in terms of electrical conductivity (EC) of a saturated paste extract based upon the increases in electrical conductivity of soil or water as salt concentration increases (McNeal, 1981; Green, 1999). EC measurements are reported in mmhos/cm or deciSiemens per meter (dS/m) (Rhoades et al, 1992). Soils with an EC > 4 dS/m are considered saline and irrigation water with an EC_e > 3 dS/m can restrict growth of several crops (Ayers and Westcot, 1985). Water is grouped in 5 classes based on conductivity levels: Class 1 – Excellent [0 – 0.250 EC or 175 ppm TDS], Class 2 – Good [0.250 – 0.750 EC or 175 – 525 ppm TDS], Class 3 – Perimissible [0.750 – 2.0 EC or 525 – 1,400 ppm TDS], Class 4-

Doubtful [2.0 – 3.0 EC or 1,400 – 2,100 ppm TDS] and Class 5 – Unsuitable [>3.0 EC or >2,100 ppm TDS] (Provin and Pitt, 2002). Salinity levels can also be expressed as Total Dissolved Salts (TDS) which is measured by evaporating filtered samples of water and weighing the amount of salt that remains. TDS is expressed in parts per million (ppm) or milligrams per liter (mg/liter) which are essentially equivalent. Common irrigation water TDS values range from 75 mg/liter to levels exceeding 4,000 mg/liter from the Pecos River near Orla, Texas (McNeal, 1981). In order to relate TDS (ppm) and EC (dS/m) to one another a general conversion is 640 ppm is equal to 1.0 dS/m (McFarland et al., 2002).

Evaluating the sodium hazard of salt-affected soils is important in order to understand the affects on plants and to develop management strategies. Two methods for quantifying sodium hazard commonly used are Sodium Absorption Ration (SAR) and Exchangeable Sodium Percentage (ESP). SAR values are expressed in milliequivalents per liter units and calculated using the following equation: $SAR = Na / [(Ca + Mg)/2]^{½}$. ESP values are expressed as a percentage and calculated using the following equation: ESP = 100 x (ESR / 1 + ESR) where ESR is the Exchangeable Sodium Ratio. For many soils from the western United States, the ESP and SAR values are numerically equal up to 25 to 30 ESP (McNeal, 1981).

There are three general types of salt affected soils: saline, sodic and saline-sodic. Saline soils (also known as “white alkali”) have an EC > 4 mmhos/cm, pH < 8.5 and Exchangeable Sodium Percentage (ESP) < 15%. Sodic soils (also known as “black alkali”) have an EC < 4 mmhos/cm, pH > 8.5 and ESP > 15%. Saline-sodic soils have an EC > 4 mmhos/cm, pH < 8.5 and ESP > 15%. (Havlin et al., 1999).

Problems Associated with Salinity

Two major problems can typically occur in crop production when irrigating with saline irrigation water: salinity hazard and sodium hazard depending upon the type of salt-affected soils (McFarland et al., 2002). These problems are based upon the chemical composition of the soil-water solution and necessitate different management strategies to remediate.

Salinity problems are very similar to drought or water stress problems because salinity affects water availability causing "physiological drought". As salinity levels in the soil solution increase the osmotic potential decreases which decreases the soil water potential (which is the combination of gravitational forces, matric potential and osmotic potential). This in turn decreases the availability of water along the soil-water gradient (higher potential in soil-water solution versus lower potential in the plant and atmosphere) (Havlin et al., 1999). As the level of salinity increases near the roots, water becomes less likely to enter the root and at times may actually be pulled out of the roots (Provin and Pitt, 2001). The effects of salinity can hamper plant germination, reduce plant growth and establishment subsequently producing low crop yields and possibly a total loss of a crop (Rhoades and Loveday, 1990). Ion toxicity attributed to Na^+ and Cl^- can also occur (Green, 1999). Also complicating salinity issues, soil-water-salinity in irrigated fields can be ten times greater at the bottom of the root zone than at the top of the root zone (Bernstein, 1975).

Sodium related problems can be very complex and problematic. Ion toxicity due to sodium can occur and cause severe limitation to plant growth. Sodic soils tend to have a very poor physical condition becoming sticky and plastic when wet and very hard when dry which in turn severly limits the hydraulic conductivity causing water infiltration and plant germination problems. These soil structure problems are caused by a high percentage of the cation exchange capacity (CEC) being occupied by Na and subsequently the dispersal of soil aggregates (Havlin et al., 1999). Accumulation of salts can occur as saline irrigation water is applied to a field. Soil salinization potential is based soley upon water quality but recent findings indicate it cannot be based only on this parameter but the spatial variability of soil salinity must also be considered (Miyamoto et al., 2005).

<u>Salt Tolerance in Plants</u>

Salt tolerance in plants varies across plant species, and it is important to understand how plants are characterized in terms of their ability to tolerate salinity. Tolerance of plants is commonly divided into 4 broad categories: Sensitive (EC $<$ 1.3 dS/m), Moderately Sensitive (EC of 1.3 - 3 dS/m), Moderately Tolerant (EC of 3 dS/m to 6 dS/M) and Tolerant (EC of 6 dS/m to 10 dS/m). EC values listed represent the salinity levels at which a 100% yield is limited.

It has been shown that glycophytes and halophytes accomplish salt tolerance through variations in the response of the plasma membrane permeability (Mansour and Salama, 2004). There are three key processes that contribute to salt tolerance at the cellular level: the establishment of cellular ion homestasis, the synthesis of compatible solutes for osmotic adjustment, and the increased ability of cells to neutralize reactive oxygen species generated during the stress response (Blumwald, 2005). Additionally, most legumes respond to saline conditions by salt exclusion or the exclusion of sodium and/or chloride from the leaves which generally lends to salt tolerance (Lauchli, 1984).

Characterizing salt tolerance in plants can be time and labor consuming as in-depth projects are required. The use of Tetrazolium testing at the biochemical level, which stains living cells, may provide a more rapid and efficient method for future salt tolerance work (Assadian et al., 2005).

<u>Salt Tolerance of Alfalfa</u>

Alfalfa has been characterized as moderately sensitive to salts with 2.0 dS/m electrical conductivity and a threshold of 1.5 bar osmotic potential of soil solution at field capacity (Maas and Hoffman, 1977). An additional 7% decrease in alfalfa yields can be expected with each mmho/cm increase in saturation extract salinity (Rawlins, 1979). In contrast, alfalfa has also been characterized as tolerant to salts with a range of EC x 10^3 values from 6.0 to 8.0 mmhos/cm at which some reduction in growth and yields can be expected (Longenecker and Lyerly, 1974).

A recent study compared alfalfa to 4 other crops [saltbush (*Atriplex spp.*), balansa clover (*Trifolium michelianum*), subclover (*Trifolium subterraneum*) and tall wheatgrass (*Thinopyrum ponticum*)], and described alfalfa as a more salt tolerant crop (Munns, 2005). Alfalfa was as tolerant as barley (*Hordeum vulgare*) and cotton (*Gossypium spp.*), however the growth of alfalfa was still retarded in saline conditions. When soils and water are both saline, it is necessary to irrigate about every other day for 6 or 7 days until alfalfa germinates and then withhold irrigation water until seedlings have several leaves or are two inches tall (Lindsey et al., 1970). Moderate yield reductions have been observed in alfalfa crops and other forages grown with irrigation of 3 to 5 mmho/cm (Miyamoto et al., 1984). The rate and final emergence of alfalfa cultivars declined when salinity of irrigation exceeded 4.3 dS/m, even though the seed germinated well in saline solutions of 28 dS/m (Assadian and Miyamoto, 1987). Alfalfa plants that were sub-irrigated with 5,000 ppm (= EC of 7.8) water had deformed cotyledons and were more chlorotic than were control plants (Johnson, 1989). Alfalfa can be established with minimal salt injury at levels up to 4.0 dS/m when seeded approximately 10 mm deep (Assadian and Miyamoto, 1987).

Mechanisms for Salt Tolerance in Alfalfa

Describing salt tolerance in alfalfa (particularly at the germination stage) has proven to be difficult due to the differences in response to salinity at different growth stages (Smith, 1998). The specific mechanisms of tolerance to salinity that are used by alfalfa are unknown (Smith, 1998). Alfalfa plants utilize salt exclusion as a mechanism to cope with salinity issues and they do exclude Na^+ but do not exclude Cl^- (Brown and Hayward, 1956; Lauchli, 1984). Alfalfa is more salt tolerant because it is able to regulate the uptake and translocation of Na^+ and Cl^- to prevent excessive accumulation of these ions in leaves (Munns, 2005).

Genetic Variability of Alfalfa

Legumes (such as alfalfa) have great genetic variability which emphasizes the importance of analyzing cultivars. Germination characters can be influenced by varieties and NaCl levels (Hefny and Dolinski, 1998). Significant differences among cultivars have been observed for ability to germinate under salt stress and subsequent selection of a cultivar (Ladak 65) at 1.75% NaCl in an agar medium resulted in a 3.75 fold increase at that salt concentration (Carlson et al., 1983). Cell culture techniques have been used to select a salt tolerant alfalfa line (W75RS) that was not affected by a salt level of 62.5 mM NaCl in nutrient solution regardless of whether callus cultures or whole plants were examined (Croughan et al., 1978; Smith and McComb 1981). In vitro selection techniques were used to evaluate callus cultures of CUF101 and CUF101S alfalfa for salt tolerance. Regeneration buds in highly saline solution revealed one somaclone (6R2IV) to have increased salt tolerance than the parent line and multiple copies of the pA9 gene versus single copies in the parent line (Safarenjad et al., 1996). This evidence indicates that salinity problems can potentially be remedied through the selection of more tolerant cultivars.

Significant differences in water use efficiency were shown between alfalfa cultivars (higher transpiration efficiency of Zhongmu No. 1 and Qinglai cultivars as compared to lower transpiration efficiency of Aohan and Shouling cultivars $p<0.01$) and may have potential in dealing with water shortage issues in the future (Li and Zhang, 2004). Research on the trait for high fibrous rooting characteristics in alfalfa has shown some potential in usefulness as a salinity stress avoidance mechanism (Vaughan et al., 2002). The popularity of biotechnology has been investigated as a means to improve salinity tolerance in alfalfa but none have successful (Smith and Dobrenz, 1987a).

CHAPTER III
SALT TOLERANCE OF GERMINATING ALFALFA SEEDS

Introduction

The first experiment evaluated plant germination under increasing levels of salt concentration after a period of seven days of exposure. This experiment utilized the screening protocol "Salt Tolerance of Germinating Alfalfa Seeds" outlined by the North American Alfalfa Improvement Conference (NAAIC) (Rumbaugh, 1991).

Materials and Methods

Thirty alfalfa cultivars were evaluated in this experiment (Table 3.1). Commercial and experimental cultivars were donated by several companies including: Pioneer, S&W Seed, Roswell Seed, America's Alfalfa, Cal West Seeds, Target Seed and Forage Genetics. A local cultivar, "Barstow Common", was also obtained from a local alfalfa producer based on its adaptability to the area and wide use by producers. Barstow Common was assumed to have been eliminated by 1962, was originally developed for the Pecos, TX area and was estimated to produce 5-6 tons of forage and up to 1,000 pounds of seed per acre (Pecos Enterprise, 2001). Additionally, four "check" cultivars, Malone and Mesa Sirsa (Tolerant) and Saranac and Rambler (Susceptible) were obtained from the USDA Agricultural Research Service (ARS) National Plant Germplasm System (NPGS) Germplasm Resource Information Network (GRIN) to be used as controls in this experiment. All alfalfa cultivars were evaluated for germination under increasing levels of salt concentration (Table 3.1).

The method used is a standard screening NAAIC protocol. The standard protocol was amended by not using the following salt concentrations: 0.75%, 1.25%, and 1.75%

(wt/wt). Twenty-five seeds from each cultivar were placed in 100-mm Petri dishes under 5 concentrations NaCl [0.00%, 0.50%, 1.00%, 1.50%, and 2.00% (wt/wt) equivalent to 0 ppm, 5,000 ppm, 10,000 ppm, 15,000 ppm and 20,000 ppm dissolved salts or 0.0 mM, 85.6 mM, 171.1 mM, 256.7 mM and 342.2 mM NaCl solution, respectively] in de-ionized water. Each Petri dish contained 4.5mL of the appropriate salt solution and each dish was sprayed with Thiram fungicide. Each cultivar was replicated twice in a randomized complete block design. The temperature was maintained at a constant 25^{o} C and seeds were evaluated 1 week after the experiment was established. Seeds were recorded as either germinated or non-germinated and data were converted to mean percent germination.

These data were then utilized to develop an IC(50) value which was used to describe the osmotic potential required to inhibit germination of 50% of the viable seeds. Calculation of the IC(50) is conducted because it is necessary to utilize a standard descriptor to put into perspective the response of the germplasm to saline solutions. For this experiment, and according to the 1991 NAAIC protocol for assessing salt tolerance of germinating alfalfa seeds, IC(50) values were used (Rumbaugh, 1991). IC(50) values reflect the osmotic potential that will inhibit 50% of seed from germination. The IC(50) values were calculated as follows. First, the salt concentrations were converted to reflect osmotic potential in the unit of megapascals (MPa) using the following equation (MPa = [0.173 – (0.0269)(mmol kg^{-1})] x 0.10) as outlined in the NAAIC procedure. The corresponding values are as follows: 0% solution = 0.0173 MPa, 0.5% solution = -0.212964 MPa, 1.00% solution = -0.442959 MPa, 1.5% solution = -0.673223 MPa and 2.00% solution = -0.903218 MPa. These values were then graphed (x-axis) with the average percentage germination (y-axis) and the data were fit with a straight linear trendline in a linear regression fashion. The IC(50) value was then estimated using the fitted line and the following equation: IC(50) = (0.5 – b)/a

Mean percentage germination was sorted by cultivar for each level of salt concentration and then subjected to analysis of variance. The p-value is noted for each level of salt concentration to indicate significant difference at alpha = 0.05 and Least Significant Difference (LSD) at the 5% level is noted if significant in the referenced Appendix Table.

Results

At the 0.00% salt solution (0 ppm) there were no significant differences in emergence among cultivars at the seven day evaluation (Table 3.2, Table A1). Given that seed germination rates were not significantly different among alfalfa cultivars at the 0.00% salt solution treatment, mean percentage germination was not adjusted.

At the 0.50% salt solution (5,000 ppm) there were significant differences in germination among cultivars at the seven day (Table A2). The following cultivars had a significantly higher mean percentage germination than did the other varieties: Pioneer 58N57, S&W9215, New Mexico Common, TS9026, CW50085, FG65T067, Ameristand 802, Ameristand 801S, Mecca, FG85M282, FG75T072, TS5005 and CW59086 (Table 3.3).

At the 1.00% salt solution (10,000 ppm) there were significant differences in germination among cultivars at the seven day evaluation (Table A3). The following cultivars had a significantly higher mean percentage germination than did the other cultivars: TS8018, CW39060, Ameristand 801S, S&W9215, TS9026, FT65T067, Ameristand 802, CW50085, S&W9720, Salado, FG75T072, CW59086, Mecca, TS9025, Barstow Common and Pioneer 58N57 (Table 3.4)

At the 1.50% salt solution (15,000 ppm) there were significant differences in germination among cultivars at the seven day evaluation (Table A4). The following cultivars had significantly higher mean percentage germination than did the other cultivars: S&W9720, CW59086, TS9025, Mecca, FG65T067, TS5016 and FG75T072 (Table 3.5).

At the 2.00% salt solution (20,000 ppm) there were significant differences in germination among cultivars at the seven day evaluation (Table A5). TS9025 had significantly higher mean percentage germination than all other cultivars at the 2.00% salt solution treatment with 94% (Table 3.6).

Utilizing the line equation the IC(50) values were calculated and ranked (Table 3.7). The cultivar with the greatest IC(50) value was TS 9025 at -26.65 MPa. Of the 34 cultivars, 12 had IC(50) values greater than -1.00. The cultivar with the lowest IC(50) value was a susceptible check, Rambler, at -0.31 MPa. Of the commercial and experimental cultivars, the greatest and lowest IC(50) values were TS9025 and FG 65HG501, respectively (Figures 3.1 and 3.2). The check cultivars with the greatest and lowest IC(50) values were Malone and Rambler, respectively (Figures 3.3 and 3.4).

Conclusion

Salt tolerance is critical in the establishment stage of plant development and this experiment clearly shows that significant differences do exist among alfalfa cultivars as salinity levels increase. Plant breeding practices that select for germination in saline conditions can provide one means of improving salt tolerance in alfalfa given the genetic variability.

Only four alfalfa cultivars were consistent in ranking in the significantly highest group at the 0.50%, 1.00% and 1.50% salt solution treatments, including: CW59086, Mecca, FG65T067 and FG75T072. However, the highest percentage germination of any cultivar at the 2.00% germination was TS9025. Given the variation among rankings at varying salt solution levels it is difficult to assess the most salt tolerant cultivar based on these data alone. This is due to the fact that germination is only one stage of growth in a plant, and how it is able to tolerate saline conditions throughout the life of the plant is also critical. Thus, it is vital to further explore the forage production of these varieties under salt stress.

Table 3.1: Source and name of alfalfa cultivars screened in experiment one

Company	**Variety**	**Fall Dormancy Rating**
Pioneer	58N57	8
S&W Seed	SW9215	9
S&W Seed	SW9720	9
America's Alfalfa	AmeriStand 801S	8
America's Alfalfa	AmeriStand 802	8
America's Alfalfa	Salado	9
Roswell Seed	Wilson	unknown
Roswell Seed	New Mexico Common	unknown
Roswell Seed	Dona Ana	7
Local Landrace	Barstow Common	unknown
Cal/West	Mecca III	9
Cal/West	CW58071	8
Cal/West	CW59060	9
Cal/West	CW50085	10
Cal/West	CW39060	9
Cal/West	CW59086	9
Target Seed	TS-5005	5
Target Seed	TS-5016	5
Target Seed	TS-7024	7
Target Seed	TS-8018	8
Target Seed	TS-8028	8
Target Seed	TS-9025	9
Target Seed	TS-9026	9
Target Seed	TS-0002	10
Forage Genetics	65HG501	6
Forage Genetics	65T067	6
Forage Genetics	75T072	7
Forage Genetics	85M282	8
Forage Genetics	91T005GT	9
Forage Genetics	R105BD101	10
Check--Tolerant	Malone	7
Check--Tolerant	Mesa Sirsa	unknown
Check--Susceptible	Saranac	unknown
Check--Susceptible	Rambler	unknown

** Check varieties obtained from the USDA Agricultural Research Service (ARS) National Plant Germplasm System (NPGS) - Germplasm Resource Information Network (GRIN)
Fall Dormancy Rating – unknown: indicates an unknown FD Rating

Table 3.2: Mean percentage germination after 7 days of exposure to 0.00% salt solution

	% NaCl				
Cultivar	**0.0%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**
Pioneer 58N57	**100[a]**	100	96	80	26
Target Seed 5005	**100[a]**	98	90	88	60
S&W SW9720	**100[a]**	96	98	96	32
Target Seed 5016	**100[a]**	96	90	92	32
Cal/West CW58071	**100[a]**	96	92	86	22
Forage Genetics 65T067	**100[a]**	100	98	94	16
Ameristand 802	**98[a]**	100	98	88	42
Ameristand 801S	**98[a]**	100	100	90	34
Dona Ana	**98[a]**	96	90	68	16
Cal/West CW59060	**98[a]**	94	92	74	12
Cal/West CW39060	**96 [a]**	96	100	88	32
Salado	**96 [a]**	94	98	82	86
Cal/West Mecca	**96 [a]**	100	96	96	32
Cal/West CW50085	**96[a]**	100	98	86	26
Target Seed 9026	**96[a]**	100	98	80	78
Cal/West CW59086	**96[a]**	98	98	96	34
S&W SW9215	**96[a]**	100	98	78	2
Target Seed 9025	**96[a]**	96	96	96	94
Forage Genetics 75T072	**96[a]**	98	98	92	18
Barstow Common	**94[a]**	94	96	76	6
Wilson	**94[a]**	90	90	58	6
Mesa Sirsa - Check	**94[a]**	94	90	78	36
Forage Genetics 85M282	**94[a]**	100	90	84	20
NM Common	**92[a]**	100	94	72	28
Target Seed 8018	**92[a]**	94	100	90	82
Forage Genetics 91T005GT	**92[a]**	94	88	78	8
Forage Genetics 65HG501	**90[a]**	90	92	50	10
Target Seed 7024	**90[a]**	90	92	82	16
Forage Genetics R105BD101	**90[a]**	90	90	84	36
Target Seed 0002	**88[a]**	68	82	78	24
Saranac - Check	**88[a]**	92	78	80	10
Target Seed 8028	**86[a]**	80	84	76	38
Rambler - Check	**84[a]**	72	22	2	0
Malone - Check	**84[a]**	92	86	74	50
Mean	**94.4**	94.1	91.1	79.8	31.3
Standard Deviation	**4.63**	7.51	13.37	17.24	24.14
P-value	**0.38**	0.01	1.22E-5	6.81E-6	7.49E-8
LSD (0.05)	**NS**	3.53	4.35	5.47	6.38

LSD (0.05) represents the Least Significant Difference at the 5% level.
NS means that there were no significant differences between varieties within that column at the 5% level
Corresponding letters (a, ab, bcd, etc) indicate values with no significant difference

Table 3.3: Mean percentage germination after 7 days of exposure to 0.50% salt solution

	% NaCl				
Cultivar	**0.0%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**
Pioneer 58N57	100	**100[a]**	96	80	26
S&W SW9215	96	**100[a]**	98	78	2
NM Common	92	**100[a]**	94	72	28
Target Seed 9026	96	**100[a]**	98	80	78
Cal/West CW50085	96	**100[a]**	98	86	26
Forage Genetics 65T067	100	**100[a]**	98	94	16
Ameristand 802	98	**100[a]**	98	88	42
Ameristand 801S	98	**100[a]**	100	90	34
Cal/West Mecca	96	**100[a]**	96	96	32
Forage Genetics 85M282	94	**100[a]**	90	84	20
Forage Genetics 75T072	96	**98[ab]**	98	92	18
Target Seed 5005	100	**98[ab]**	90	88	60
Cal/West CW59086	96	**98[ab]**	98	96	34
Dona Ana	98	**96[bc]**	90	68	16
Target Seed 5016	100	**96[bc]**	90	92	32
Cal/West CW58071	100	**96[bc]**	92	86	22
S&W SW9720	100	**96[bc]**	98	96	32
Cal/West CW39060	96	**96[bc]**	100	88	32
Target Seed 9025	96	**96[bc]**	96	96	94
Barstow Common	94	**94[cd]**	96	76	6
Forage Genetics 91T005GT	92	**94[cd]**	88	78	8
Mesa Sirsa - Check	94	**94[cd]**	90	78	36
Salado	96	**94[cd]**	98	82	86
Cal/West CW59060	98	**94[cd]**	92	74	12
Target Seed 8018	92	**94[cd]**	100	90	82
Saranac – Check	88	**92[de]**	78	80	10
Malone - Check	84	**92[de]**	86	74	50
Wilson	94	**90[e]**	90	58	6
Forage Genetics 65HG501	90	**90[e]**	92	50	10
Target Seed 7024	90	**90[e]**	92	82	16
Forage Genetics R105BD101	90	**90[e]**	90	84	36
Target Seed 8028	86	**80[f]**	84	76	38
Rambler - Check	84	**72[g]**	22	2	0
Target Seed 0002	88	**68[h]**	82	78	24
Mean	94.4	**94.1**	91.1	79.8	31.3
Standard Deviation	4.63	**7.51**	13.37	17.24	24.14
P-value	0.38	**0.01**	1.22E-5	6.81E-6	7.49E-8
LSD (0.05)	NS	**3.53**	4.35	5.47	6.38

LSD (0.05) represents the Least Significant Difference at the 5% level.
NS means that there were no significant differences between varieties within that column at the 5% level
Corresponding letters (a, ab, bcd, etc) indicate values with no significant difference

Table 3.4: Mean percentage germination after 7 days of exposure to 1.00% salt solution

Cultivar	% NaCl				
	0.0%	**0.5%**	**1.0%**	**1.5%**	**2.0%**
Target Seed 8018	92	94	**100**[a]	90	82
Cal/West CW39060	96	96	**100**[a]	88	32
Ameristand 801S	98	100	**100**[a]	90	34
S&W SW9215	96	100	**98**[ab]	78	2
Target Seed 9026	96	100	**98**[ab]	80	78
Forage Genetics 65T067	100	100	**98**[ab]	94	16
Ameristand 802	98	100	**98**[ab]	88	42
Cal/West CW50085	96	100	**98**[ab]	86	26
S&W SW9720	100	96	**98**[ab]	96	32
Salado	96	94	**98**[ab]	82	86
Forage Genetics 75T072	96	98	**98**[ab]	92	18
Cal/West CW59086	96	98	**98**[ab]	96	34
Cal/West Mecca	96	100	**96**[abc]	96	32
Target Seed 9025	96	96	**96**[abc]	96	94
Barstow Common	94	94	**96**[abc]	76	6
Pioneer 58N57	100	100	**96**[abc]	80	26
NM Common	92	100	**94**[bcd]	72	28
Cal/West CW58071	100	96	**92**[cde]	86	22
Target Seed 7024	90	90	**92**[cde]	82	16
Forage Genetics 65HG501	90	90	**92**[cde]	50	10
Cal/West CW59060	98	94	**92**[cde]	74	12
Dona Ana	98	96	**90**[def]	68	16
Forage Genetics 85M282	94	100	**90**[def]	84	20
Target Seed 5005	100	98	**90**[def]	88	60
Target Seed 5016	100	96	**90**[def]	92	32
Forage Genetics R105BD101	90	90	**90**[def]	84	36
Mesa Sirsa - Check	94	94	**90**[def]	78	36
Wilson	94	90	**90**[def]	58	6
Forage Genetics 91T005GT	92	94	**88**[efg]	78	8
Malone – Check	84	92	**86**[fgh]	74	50
Target Seed 8028	86	80	**84**[gh]	76	38
Target Seed 0002	88	68	**82**[hi]	78	24
Saranac - Check	88	92	**78**[i]	80	10
Rambler - Check	84	72	**22**[j]	2	0
Mean	94.4	94.1	**91.1**	79.8	31.3
Standard Deviation	4.63	7.51	**13.37**	17.24	24.14
P-value	0.38	0.01	**1.22E-5**	6.81E-6	7.49E-8
LSD (0.05)	NS	3.53	**4.35**	5.47	6.38

LSD (0.05) represents the Least Significant Difference at the 5% level.
NS means that there were no significant differences between varieties within that column at the 5% level
Corresponding letters (a, ab, bcd, etc) indicate values with no significant difference

Table 3.5: Mean percentage germination after 7 days of exposure to 1.50% salt solution

	% NaCl				
Cultivar	**0.0%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**
S&W SW9720	100	96	98	**96**[a]	32
Cal/West CW59086	96	98	98	**96**[a]	34
Target Seed 9025	96	96	96	**96**[a]	94
Cal/West Mecca	96	100	96	**96**[a]	32
Forage Genetics 65T067	100	100	98	**94**[ab]	16
Target Seed 5016	100	96	90	**92**[abc]	32
Forage Genetics 75T072	96	98	98	**92**[abc]	18
Ameristand 801S	98	100	100	**90**[bcd]	34
Target Seed 8018	92	94	100	**90**[bcd]	82
Cal/West CW39060	96	96	100	**88**[cde]	32
Ameristand 802	98	100	98	**88**[cde]	42
Target Seed 5005	100	98	90	**88**[cde]	60
Cal/West CW50085	96	100	98	**86**[def]	26
Cal/West CW58071	100	96	92	**86**[def]	22
Forage Genetics R105BD101	90	90	90	**84**[efg]	36
Forage Genetics 85M282	94	100	90	**84**[efg]	20
Salado	96	94	98	**82**[fgh]	86
Target Seed 7024	90	90	92	**82**[fgh]	16
Target Seed 9026	96	100	98	**80**[ghi]	78
Saranac – Check	88	92	78	**80**[ghi]	10
Pioneer 58N57	100	100	96	**80**[ghi]	26
Mesa Sirsa - Check	94	94	90	**78**[hij]	36
Forage Genetics 91T005GT	92	94	88	**78**[hij]	8
Target Seed 0002	88	68	82	**78**[hij]	24
S&W SW9215	96	100	98	**78**[hij]	2
Barstow Common	94	94	96	**76**[ijk]	6
Target Seed 8028	86	80	84	**76**[ijk]	38
Cal/West CW59060	98	94	92	**74**[jk]	12
Malone - Check	84	92	86	**74**[jk]	50
NM Common	92	100	94	**72**[kl]	28
Dona Ana	98	96	90	**68**[l]	16
Wilson	94	90	90	**58**[m]	6
Forage Genetics 65HG501	90	90	92	**50**[n]	10
Rambler - Check	84	72	22	**2**[o]	0
Mean	94.4	94.1	91.1	**79.8**	31.3
Standard Deviation	4.63	7.51	13.37	**17.24**	24.14
P-value	0.38	0.01	1.22E-5	**6.81E-6**	7.49E-8
LSD (0.05)	NS	3.53	4.35	**5.47**	6.38

LSD (0.05) represents the Least Significant Difference at the 5% level.
NS means that there were no significant differences between varieties within that column at the 5% level
Corresponding letters (a, ab, bcd, etc) indicate values with no significant difference

Table 3.6: Mean percentage germination after 7 days of exposure to 2.00% salt solution

	% NaCl				
Cultivar	**0.0%**	**0.5%**	**1.0%**	**1.5%**	**2.0%**
Target Seed 9025	96	96	96	96	**94[a]**
Salado	96	94	98	82	**86[b]**
Target Seed 8018	92	94	100	90	**82[bc]**
Target Seed 9026	96	100	98	80	**78[c]**
Target Seed 5005	100	98	90	88	**60[d]**
Malone - Check	84	92	86	74	**50[e]**
Ameristand 802	98	100	98	88	**42[fg]**
Target Seed 8028	86	80	84	76	**38[gh]**
Forage Genetics R105BD101	90	90	90	84	**36[gh]**
Mesa Sirsa – Check	94	94	90	78	**36[gh]**
Ameristand 801S	98	100	100	90	**34[hi]**
Cal/West CW59086	96	98	98	96	**34[hi]**
Cal/West Mecca	96	100	96	96	**32[hij]**
S&W SW9720	100	96	98	96	**32[hij]**
Target Seed 5016	100	96	90	92	**32[hij]**
Cal/West CW39060	96	96	100	88	**32[hij]**
NM Common	92	100	94	72	**28[ijk]**
Cal/West CW50085	96	100	98	86	**26[jkl]**
Pioneer 58N57	100	100	96	80	**26[jkl]**
Target Seed 0002	88	68	82	78	**24[klm]**
Cal/West CW58071	100	96	92	86	**22[klmn]**
Forage Genetics 85M282	94	100	90	84	**20[lmn]**
Forage Genetics 75T072	96	98	98	92	**18[mno]**
Dona Ana	98	96	90	68	**16[nop]**
Target Seed 7024	90	90	92	82	**16[nop]**
Forage Genetics 65T067	100	100	98	94	**16[nop]**
Cal/West CW59060	98	94	92	74	**12[opq]**
Saranac - Check	88	92	78	80	**10[pq]**
Forage Genetics 65HG501	90	90	92	50	**10[pq]**
Forage Genetics 91T005GT	92	94	88	78	**8[qr]**
Wilson	94	90	90	58	**6[qrs]**
Barstow Common	94	94	96	76	**6[qrs]**
S&W SW9215	96	100	98	78	**2[rs]**
Rambler - Check	84	72	22	2	**0[s]**
Mean	94.4	94.1	91.1	79.8	**31.3**
Standard Deviation	4.63	7.51	13.37	17.24	**24.14**
P-value	0.38	0.01	1.22E-5	6.81E-6	**7.49E-8**
LSD (0.05)	NS	3.53	4.35	5.47	**6.38**

LSD (0.05) represents the Least Significant Difference at the 5% level.
NS means that there were no significant differences between varieties within that column at the 5% level
Corresponding letters (a, ab, bcd, etc) indicate values with no significant difference

Table 3.7: Osmotic potential [IC(50) value] inhibiting germination of 50% of seeds and the linear regression line equation for 36 alfalfa cultivars

Cultivar	IC(50) (MPa)	Linear Regression Line Equation
Target Seed 9025	-26.65	y=0.0174x+0.9637
Target Seed 8018	-4.43	y=0.1043x+0.9622
Salado	-3.40	y=0.1391x+0.9736
Target Seed 9026	-2.10	y=0.2433x+1.0118
Target Seed 5005	-1.39	y=0.3911x+1.0452
Malone (Tolerant CK)	-1.17	y=0.373x+0.9375
Ameristand 802	-1.10	y=0.5388x+1.0907
Cal/West CW59086	-1.07	y=0.5475x+1.0865
Cal/West Mecca	-1.04	y=0.5735x+1.0941
S&W SW9720	-1.03	y=0.5909x+1.1058
Ameristand 801S	-1.02	y=0.5996x+1.1096
Forage Genetics R105BD101	-1.01	y=0.4953x+0.9994
Cal/West CW39060	-0.99	y=0.5909x+1.0858
Target Seed 5016	-0.97	y=0.6083x+1.0895
Target Seed 8028	-0.97	y=0.4345x+0.9205
Mesa Sirsa (Tolerant CK)	-0.94	y=0.5736x+1.0381
Cal/West CW50085	-0.91	y=0.6691x+1.1084
Forage Genetics 75T072	-0.87	y=0.7039x+1.1158
Forage Genetics 65T067	-0.86	y=0.756x+1.1509
Pioneer 58N57	-0.86	y=0.73x+1.274
Cal/West CW58071	-0.85	y=0.7213x+1.1115
NM Common	-0.84	y=0.6778x+1.0723
Forage Genetics 85M282	-0.83	y=0.7126x+1.0917
Target Seed 7024	-0.80	y=0.6778x+1.0403
Target Seed 0002	-0.79	y=0.5127x+0.9071
Cal/West CW59060	-0.73	y=0.8343x+1.1096
Dona Ana	-0.73	y=0.8343x+1.1056
Barstow Common	-0.72	y=0.8429x+1.1054
Forage Genetics 91T005GT	-0.72	y=0.7995x+1.0742
S&W SW9215	-0.71	y=0.9125x+1.1522
Saranac (Susceptible CK)	-0.71	y=0.7299x+1.0194
Wilson	-0.64	y=0.9038x+1.0764
Forage Genetics 65HG501	-0.63	y=0.8691x+1.049
Rambler (Susceptible CK)	-0.31	y=1.0342x+0.8182
AVERAGE	**-1.85**	**y=0.6101x+1.0515**

*CK denotes a check cultivar and the expected response to saline conditions (tolerant or susceptible)

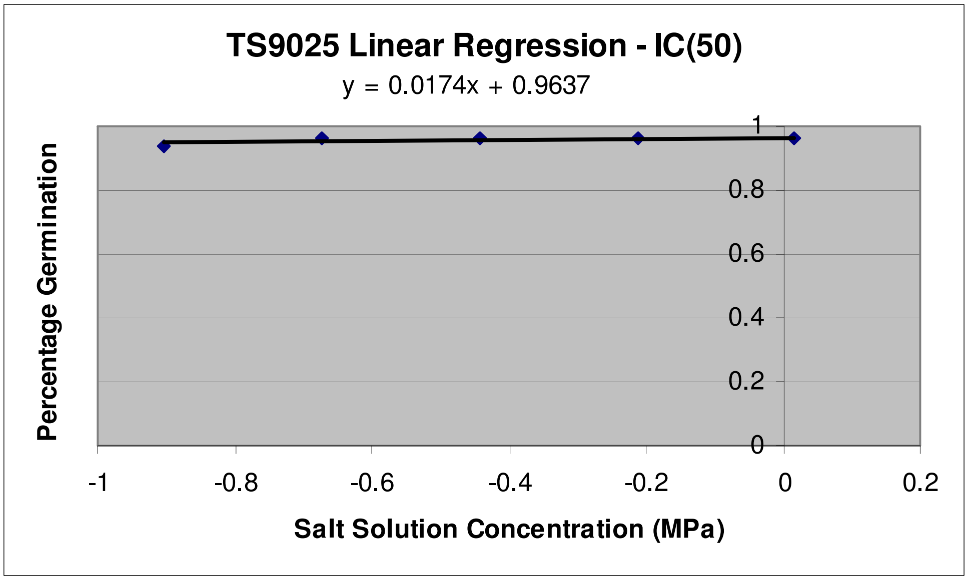

Figure 3.1: Linear regression line for TS9025 used to determine the IC(50) value (-26.65 MPa)

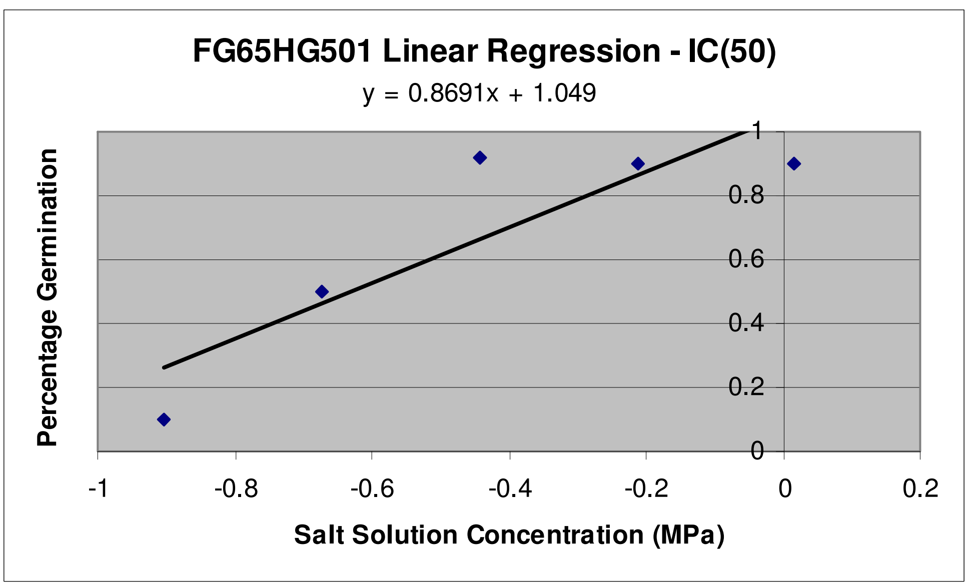

Figure 3.2: Linear regression line for FG65HG501 used to determine the IC(50) value (-0.63 MPa)

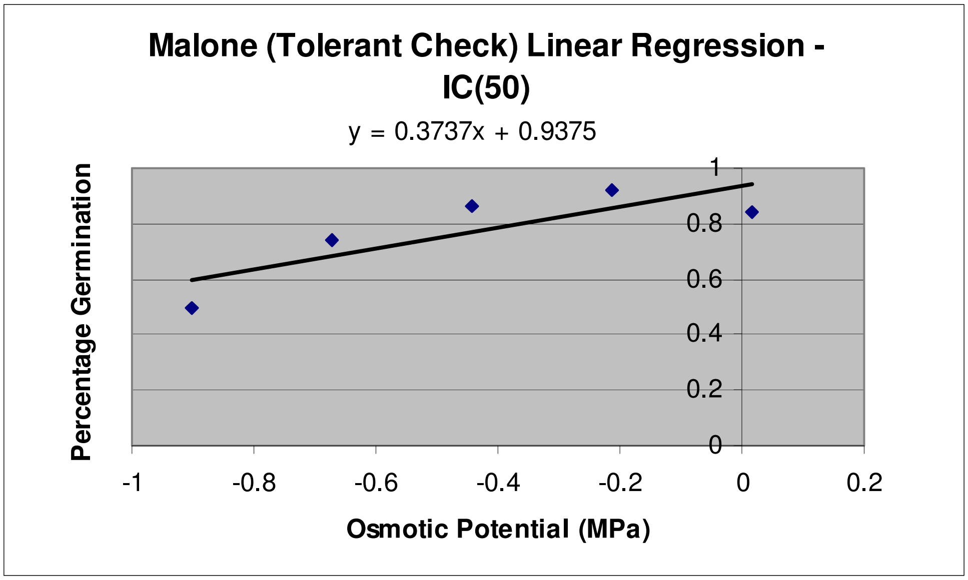

Figure 3.3: Linear regression line for Tolerant Check Cultivar (Malone) used to determine the IC(50) value (-1.17 MPa)

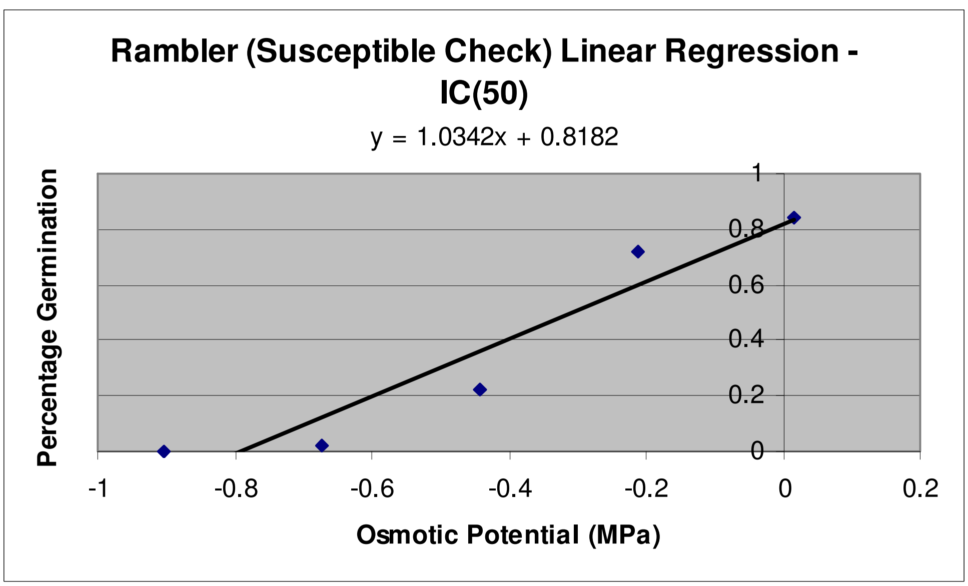

Figure 3.4: Linear regression line for Susceptible Check Cultivar (Rambler) used to determine the IC(50) value (-0.31 MPa)

CHAPTER IV
FORAGE PRODUCTION OF ALFALFA UNDER SALT STRESS

Introduction

The second experiment evaluated forage production under increasing levels of salt concentration over a period of four harvests. This experiment utilized the standard screening protocol "Forage Production Under Salt Stress" outlined by the North American Alfalfa Improvement Conference (Smith, 1991). Salinity tolerance is evaluated on a relative basis in this experiment by comparing yields under a non-saline treatment to yields under a saline treatment over time.

Materials and Methods

The same thirty alfalfa cultivars (Table 4.1) were planted in 4 cm x 20 cm cone-tainers (3 to 5 seeds per cone-tainer), covered with 10 mm sand and thinned to 1 seedling after 14 days. All cone-tainers were irrigated for the first 14 days with 0.25X Hoaglands solution to ensure plant establishment (California Fertilizer Association, 1985). One group received a non-saline irrigation treatment of the Hoagland's solution for the remainder of the experiment. The other group was irrigated with the Hoaglands solution and 3.5g/L NaCl (60mM) for the remainder of the experiment. Herbage was harvested (3 cm above the soil surface) at 50 days after planting (DAP) (referred to as Cut 1) and discarded. Herbage was then harvested at 30 day intervals and fresh forage weight recorded for each plant. A total of three harvests (referred to as Cut 2, Cut 3 and Cut 4) at thirty day intervals were completed after the initial 50 DAP harvest and disposal. The study was replicated twice with 28 entries per cultivar per replication per treatment (non-saline and saline) in a randomized design. Replication over time was also incorporated into the experimental design with the three recorded harvest dates.

Two test cultivars were also screened as the control population in this experiment. The "Tolerant" cultivar was AZ-90NDC-ST and the "Susceptible" cultivar was AZ-88NDC. These cultivars were obtained from Dr. Steve Smith of the University of Arizona Plant Science Department and used as controls in this experiment

Trimax insecticide (active ingredient Imidacloprid 40.7%; Research Triangle Park, NC), a Bayer Crop Science product with a mode of action of nicotinic acetylcholine receptor antagonist, was applied to all plants (both replication 1 and replication 2) on August 28, 2006 at 1 mL per gallon to control cowpea aphid found on plants in the greenhouse.

Forage production under saline stress is expressed as mean weight herbage produced with saline irrigation as percentage of that produced under non-saline conditions. The corresponding value is derived by comparing the production values of the salt set divided by the control set which yields a ratio or the Salt Control Ratio (SCR). SCR values were then subjected to analysis of variance. The p-value is noted to indicate significant difference at alpha = 0.05 and Least Significant Difference (LSD) at the 5% level is noted if significant. SCR values were then ranked from highest to lowest and arctransformed for data analysis as suggested by the NAAIC screening protocol (Table 4.2). Transforming data is useful when data may not be normally distributed. These data were arctransformed by calculating the arcsine of the square root of the SCR values derived in Microsoft Excel with the following formula = ASIN(SQRT(SCR Value)) (McDonald, 2008).

Results

The average yield under non-saline conditions and average yield under saline conditions are provided in Table 4.2 and Figure 4.1. The range of average non-saline yields was 3.10 grams to 2.31 grams with the average being 2.75 grams. The range of average saline yields 2.83 grams to 1.80 grams with the average being 2.29 grams. The range of SCR values for the thirty two screened alfalfa cultivars was 0.69 to 0.95 with the average value being 0.841 (Table 4.2). There were no significant differences in raw or transformed SCR values among the 32 cultivars at Cut 2, Cut 3 or Cut 4 when subject to analysis of variance (Tables A6 – A11).

SCR values were then evaluated for statistical differences using the three harvests as replications over time. Significant differences in raw and transformed SCR values were found between cultivars with Cut 2, Cut 3 and Cut 4 serving as replications over time (Tables A12, Table A13 and Figure 4.1). The following cultivars exhibited significantly higher SCR values than all others: Check Tolerant AZ-90NDC-ST, Salado, FG85M282, Pioneer 58N57, CW50085, SW9215, TS8028, CW59060, CW58071, SW9720, FG65T067 and FG75T072. The following cultivars exhibited significantly higher arctransformed SCR values than all others: Check Tolerant AZ-90NDC-ST, Salado, FG85M282, Pioneer 58N57, CW50085, SW9215 and TS8028 (Table 4.2).

Conclusion

Given that SCR values are typically less than 1.0 and can be utilized to estimate re-growth tolerance under continuing salt stress, the data generated in this experiment is useful and relevant. This experiment provides data that are indicative of salt stress over time as salt loading occurs and a plant responds physiologically to heightened saline conditions. Additionally, if SCR values are assumed to reflect re-growth tolerance, the factor of replication over time is critical. SCR values are a screening approach that seeks to ascertain the effect of cultivar response to salinity under controlled conditions to provide en masse screening of a large number of cultivars in a timely manner (as opposed to more expensive and time-demanding initial screening in the field where less control of saline conditions can occur). This information can allow plant breeders and researchers to continue to select for those differences in future experiments and attempt to utilize those trends in developing future salt tolerant cultivars.

Table 4.1: Source and name of alfalfa cultivars screened in experiment two

Company	**Variety**	**Fall Dormancy Rating**
Pioneer	58N57	8
S&W Seed	SW9215	9
S&W Seed	SW9720	9
America's Alfalfa	AmeriStand 801S	8
America's Alfalfa	AmeriStand 802	8
America's Alfalfa	Salado	9
Roswell Seed	Wilson	6
Roswell Seed	New Mexico Common	unknown
Roswell Seed	Dona Ana	7
Local Landrace	Barstow Common	unknown
Cal/West	Mecca III	9
Cal/West	CW58071	8
Cal/West	CW59060	9
Cal/West	CW50085	10
Cal/West	CW39060	9
Cal/West	CW59086	9
Target Seed	TS-5005	5
Target Seed	TS-5016	5
Target Seed	TS-7024	7
Target Seed	TS-8018	8
Target Seed	TS-8028	8
Target Seed	TS-9025	9
Target Seed	TS-9026	9
Target Seed	TS-0002	10
Forage Genetics	65HG501	6
Forage Genetics	65T067	6
Forage Genetics	75T072	7
Forage Genetics	85M282	8
Forage Genetics	91T005GT	9
Forage Genetics	R105BD101	10
Check--Tolerant**	AZ-90NDC-ST	9
Check--Susceptible**	AZ-88NDC	8

** Check varieties obtained from Dr. Steve Smith, University of Arizona Plant Science Department

Fall Dormancy Rating – unknown: indicates an unknown FD Rating

Table 4.2: Salt Control Ratio (SCR) values ranked from highest to lowest

Rank	Cultivar	SCR	ArcSine	AVG Non Saline Yield (grams)	AVG Saline Yield (grams)
1	Check Tolerant AZ-90NDC-ST	0.95^{a}	1.35^{a}	2.60	2.48
2	America's Alfalfa Salado	0.95^{a}	1.34^{ab}	3.10	2.83
3	Forage Genetics 85M282	0.94^{a}	1.32^{abc}	2.35	2.20
4	Pioneer 58N57	0.93^{ab}	1.31^{abc}	2.72	2.55
5	Cal/West CW50085	0.93^{ab}	1.30^{abc}	2.58	2.39
6	S&W SW9215	0.91^{abc}	1.27^{abcd}	2.75	2.52
7	Target Seed TS-8028	0.91^{abc}	1.27^{abcd}	2.64	2.38
8	Cal/West CW59060	0.91^{abc}	1.26^{bcd}	2.72	2.45
9	Cal/West CW58071	0.90^{abc}	1.25^{cde}	2.73	2.46
10	S&W SW9720	0.90^{abc}	1.25^{cde}	2.84	2.54
11	Forage Genetics 65T067	0.90^{abc}	1.24^{cde}	2.31	2.05
12	Forage Genetics 75T072	0.90^{abc}	1.24^{cde}	2.48	2.22
13	Target Seed TS-7024	0.87^{bcd}	1.20^{def}	2.59	2.26
14	Target Seed TS-8018	0.87^{bcd}	1.20^{def}	2.64	2.29
15	Roswell Seed NM Common	0.85^{cde}	1.17^{efg}	2.62	2.21
16	Forage Genetics 65HG501	0.83^{def}	1.14^{fgh}	2.54	2.10
17	Target Seed TS-5005	0.82^{def}	1.13^{fghi}	2.71	2.22
18	Local Barstow Common	0.81^{defg}	1.12^{fghij}	2.66	2.14
19	Cal/West CW59086	0.81^{defg}	1.11^{ghij}	3.08	2.47
20	Target Seed TS-0002	0.80^{efgh}	1.11^{ghij}	2.90	2.29
21	Forage Genetics91T005GT	0.80^{efgh}	1.11^{ghij}	2.82	2.24
22	Cal/West Mecca III	0.80^{efgh}	1.11^{ghij}	2.80	2.24
23	Forage GeneticsR105BD101	0.80^{efgh}	1.10^{ghij}	2.77	2.16
24	Target Seed TS-9026	0.80^{efgh}	1.10^{ghij}	2.98	2.33
25	AmeriStand 801S	0.79^{efgh}	1.10^{ghij}	3.08	2.43
26	Target Seed TS-5016	0.79^{efgh}	1.09^{ghij}	2.71	2.13
27	Roswell Seed Dona Ana	0.79^{efgh}	1.09^{ghij}	2.80	2.20
28	AmeriStand 802	0.76^{fgh}	1.06^{hijk}	3.12	2.35
29	Roswell Seed Wilson	0.75^{ghi}	1.05^{ijk}	2.86	2.14
30	Cal/West CW39060	0.74^{hi}	1.04^{jk}	2.99	2.17
31	Target Seed TS-9025	0.74^{hi}	1.04^{jk}	2.97	2.16
32	Check Susceptible AZ-88NDC	0.69^{i}	0.98^{k}	2.70	1.80
	Average	0.84	1.17	2.75	2.29
	P-value	0.00	0.00		
	LSD (0.05)	0.06	0.08		
	% CV	10.7%	10.4%		

SCR Value – Salt Control Ratio (average yield under saline versus the average yield under non-saline)
Arcsine – arctransformed SCR values via equation = ASIN(SQRT(SCR Value)) (McDonald, 2008)
LSD (0.05) – indicates differences between cultivars at the 5% level; % CV – Coeficient of Variation indicates variation within data; Corresponding letters (a, ab, bc, etc) indicate values with no significant difference in that column

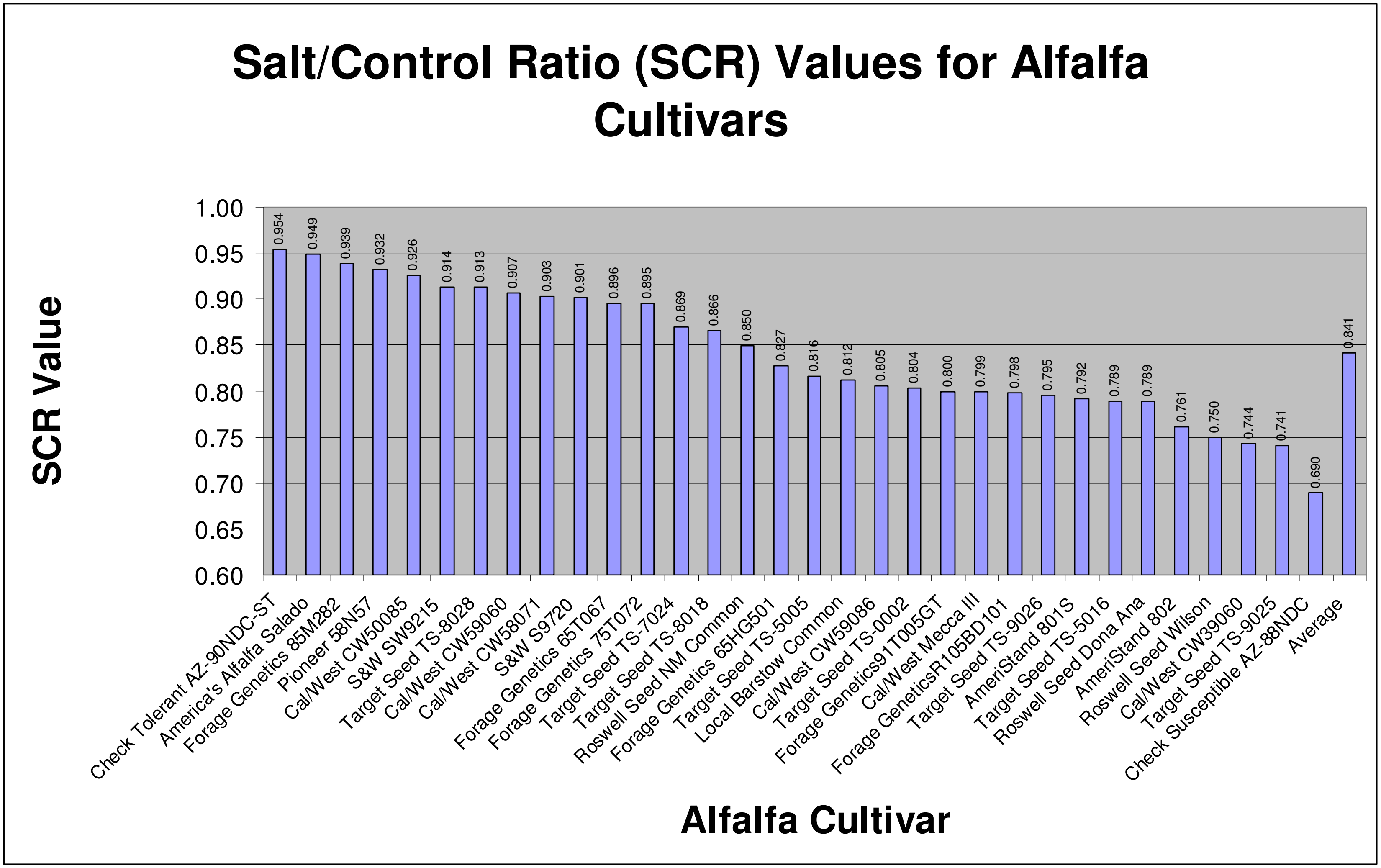

Figure 4.1: Salt Control Ratio (SCR) values line graphed

CHAPTER V
ALFALFA PRODUCTION UNDER SALINE FIELD CONDITIONS

Introduction

The third experiment evaluated forage production under saline field conditions that exist at the Texas AgriLife Research Station west of Pecos, TX. This station is located in the northern portion of the Chihuahuan desert in the Trans-Pecos region of Texas. The station is located approximately 18 miles west of the Pecos River in Reeves County.

Study Site

The soil is a Hoban silty clay loam (fine-silty, mixed, thermic Ustolic Calcuorthids) with 1.7% organic matter (USDA, 1980). Soil samples from the plots were collected in early 2007 using a metal soil probe down to a depth of six inches. Each of the 48 field plots was sampled and analyzed individually. Texas AgriLife Extension Soil, Water and Forage Testing Laboratory in College Station, Texas performed the soil analysis. Soil test data averages among the plots included: pH - 8.9, Nitrate – 11.6 ppm, Phosphorous – 42.5 ppm, Potassium – 525 ppm, Calcium – 23,397 ppm, Magnesium – 550 ppm, Sulfur – 301 ppm and Sodium – 1,502 ppm.

The average soil salinity was 0.899 mmho/com using a 1:2 soil:water extraction method (Texas Cooperative Extension, 2005). Recommendations from the laboratory suggested salinity levels were becoming elevated and to monitor or remove salts with 10-15 inches of clean leach water. Variation in values based upon analysis method (saturated paste extract versus soil:water dilution methods) and soil texture demands that a conversion to a saturated paste extract value should be noted in order to correlate electrical conductivity values with other methods (Scianna et al., 2007; Graaff and Patterson, 2001). According to the USDA Handbook 60 recommendations, the following formula [SP = 3.00 (1:1)] yields a value of 5.394 dS/m [equivalency to a 1:2 would be SP = 6.00 (1:2)] (USDA, 1954). According to the more recent research conducted by Oklahoma State University in 2005, the following formula [SP = 1.85 (1:1)] yields a value of 3.326 dS/m [equivalency to a 1:2 would be SP = 3.7 (1:1)] (Zhang et al., 2005). Low initial soil salinity values may be attributed to the cropping history of the field which had not been irrigated in 2 years and the last crop was cotton. Statistical analysis of the field plot salinity values showed no statistical differences across the field or block (Table A14). Annual soil testing has been implemented to track salinity changes in the field over time.

The average temperature at the research station is 8^{o}C in winter and the average daily minimum -2^{o}C. The average temperature is 28^{o}C in summer, and the average maximum is 37^{o}C. Annual precipitation averages 218 mm with 70% occuring from April through September, which will include the growing season for most crops.

<u>Materials and Methods</u>

Twelve alfalfa cultivars were selected from Experiment 1: *Salt Tolerance of Germinating Alfalfa Seeds* and Experiment 2: *Forage Production Under Salt Stress* based upon performance in those trials, relevance to area producers and to provide representation from the cooperating seed companies. Barstow Common was also included to provide information on a locally adapted cultivar. All cultivars were planted in a randomized complete block design with four replications. Plots were hand sown with a single-row garden seeder on October 5, 2006 at a seeding rate of 36 pounds of seed per acre with 8 rows per plot. Plot size was 6 feet by 20 feet (120 square feet) with 3 foot alleys between replications. A six to ten foot border was planted around the plots to prevent any border effect. Checks were laser leveled to ensure even flow and distribution of flood irrigation water across plots. Plots were flood irrigated at approximately 14 day intervals (immediately following a harvest and 14 days later). The well used for irrigation is estimated to produce 700 gallons per minute. The check which held the plots was irrigated for 60 minutes and thus ~42,000 gallons of water was applied at every flood irrigation.

An irrigation sample was analyzed in March 2007 at the Texas AgriLife Extension Soil, Water and Forage Testing Laboratory in College Station, Texas. Results from analysis (including criteria, units and limitation rating) included: Total Dissolved Salts (TDS) – 2,953 ppm [very limiting], Conductivity – 4,060 umhos/cm [very limiting], Sodium Absorption Ratio (SAR) – 11.7 [limiting], pH – 7.10 [acceptable], Sodium (Na^+) – 702 ppm [very limiting], Chloride (Cl^-) – 938 ppm [very limiting], Boron (B) – 0.59 ppm [acceptable], Sulfate (SO^4) – 840 ppm [limiting], Nitrate – 1.42 ppm [acceptable], Phosphorous (P) – 0.23 ppm [acceptable], Calcium (Ca) – 192 ppm [limiting/acceptable] and Charge Balance (cation/anion * 100) – 93.

The field trials were evaluated for the 2007 and 2008 growing seasons. Plots were harvested at 28-day intervals and fresh forage weights recorded. Plots were harvested at a 4 inch stubble height using a self propelled sickle bar mower for the May 7, June 4, July 2 harvests and approximately half of the July 30 harvest. However, due to mechanical problems the remainder of the plots for July 30th harvest and the remainder of the harvest dates in 2007 and 2008 were accomplished using a New Holland 479 Model swather pulled by a John Deere 7520 tractor at a 5 inch stubble height.

Subsamples from each plot were collected, stored in paper bags and weights recorded. Samples were then air dried and weights again recorded to obtain percentage moisture which was used to calculate the dry forage weight of each plot.

2007 Harvest

Plots were harvested seven times in 2007 on the following dates: May 7, June 4, July 2, July 30, August 27, September 24 and November 5. The first six harvests were all made at approximately 28-day intervals. The time period between the September 24 harvest and November 5 harvest (the sixth harvest and the seventh and final harvest) was 42 days. Sub-samples from each plot were taken, air-dried and re-weighed to calculate percent dry matter and provide dry forage yields. Soil samples were collected and analyzed and P_2O_5 was applied at 20 pounds per acre on December 17, 2007.

Field plots were flood irrigated beginning on March 16, 2007 and at approximately every 14 days throughout the growing season, with the last flood irrigation application being conducted on October 15, 2007. In 2007 a total of 37.2 inches of irrigation water was applied. 13.5 inches of rainfall were received in 2007 with the majority falling in the spring (March, April and May) and the early fall (August and September). The total moisture received from a combination of flood irrigation and rainfall in 2007 was approximately 50.7 inches.

Each harvest was subjected to statistical analysis to determine the significance of yield differences at each individual date and for total yield. All data were subjected to analysis of variance and alpha = 0.05 using the General Linear Model for repeated measures to account for the randomized complete block design. Means were separated using Least Significant Difference (LSD) and Coefficient of Variation (%CV) determined (Table 5.1).

<u>*2008 Harvest*</u>

Plots were harvested 6 times in 2008 on the following dates: April 21, May 19, June 13, July 15, August 11 and September 8. All harvests were made at approximately 28 day intervals except for the June 13 harvest which was made at 25 days and the July 15 harvest which was made at 32 days. All harvests were accomplished similar to 2007. Sub-samples from each plot were taken, air-dried and re-weighed to calculate percent dry matter and provide dry forage yields. Root rot injury became evident in July and significantly affected six of the plots in the trial. Those plots were dropped from yield data from that date forward to avoiding skewing statistical analysis. By August, root rot injury had spread to three additional plots, raising the total of affected plots to nine. Additionally, an infestation of corn earworms occurred in late July and an application of Hero (active ingredient Zeta-Cypermethrin 3.75% and Bifenthrin 11.25%; Philadelphia, PA) insecticide was made on August 6, 2008 at a rate of 300 mL per acre for control.

Field plots were flood irrigated beginning on March 26, 2008 and at approximately every 14 days throughout the growing season, with the last flood irrigation application (for the purpose of this research project) being conducted on September 2, 2008. In 2008, a total of 34.1 inches of irrigation water was applied. 4.8 inches of rainfall were received in 2008 (records only through September of 2008) with the majority falling in the spring and summer (May, June, July and August). The total moisture received (through September 8, 2008) from a combination of flood irrigation and rainfall in 2008, was approximately 38.9 inches.

Each harvest was subjected to statistical analysis to determine the significance of yield differences at each individual date and for total yield. All data were subjected to analysis of variance and alpha = 0.05 using the General Linear Model for repeated measures to account for the randomized complete block design. Means were separated using Least Significant Difference (LSD) and Coefficient of Variation (%CV) determined (Table 5.2).

Results

2007 Harvest

At the first harvest, conducted May 7, 2007, the highest yielding variety was AmeriStand 802 at 1.05 tons of dry forage per acre. The lowest yielding variety was CW59086 at 0.62 tons of dry forage per acre. The range between high and low yields was 0.43 and the mean was 0.75. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A15).

In the second harvest, conducted June 4, 2007, the highest yielding variety was AmeriStand 802 at 0.89 tons of dry forage per acre. The lowest yielding varieties were TS8028 at 0.62 tons of dry forage per acre and Pioneer 58N57 at 0.62 pounds of dry forage per acre. The range between high and low yields was 0.27 and the mean was 0.73. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A16).

In the third harvest, conducted July 2, 2007, the highest yielding variety was AmeriStand 802 at 1.16 tons of dry forage per acre. The lowest yielding variety was Pioneer 58N57 at 0.82 tons of dry forage per acre. The range between high and low yields was 0.34 and the mean was 0.95. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A17).

In the fourth harvest, conducted July 30, 2007, the highest yielding variety was AmeriStand 802 at 1.23 tons of dry forage per acre. The lowest yielding variety was Pioneer 58N57 at 0.89 tons of dry forage per acre. The range between high and low yields was 0.34 and the mean was 1.06. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A18).

In the fifth harvest, conducted August 27, 2007, the highest yielding variety was AmeriStand 801S at 1.04 tons of dry forage per acre. The lowest yielding variety was TS0002 at 0.70 tons of dry forage per acre. The range between high and low yields was 0.34 and the mean was 0.93. When subjected to analysis of variance there were significant differences among yields by cultivar at this harvest (Table A19).

In the sixth harvest, conducted September 24, 2007, the highest yielding variety was SW9720 at 0.85 tons of dry forage per acre. The lowest yielding variety was Pioneer 58N57 at 0.63 tons of dry forage per acre. The range between high and low yields was 0.22 and the mean was 0.74. When subjected to analysis of variance there were significant differences among yields by cultivar at this harvest (Table A20).

In the seventh and final harvest for the year, conducted November 5, 2007, the highest yielding variety was AmeriStand 802 at 0.85 tons of dry forage per acre. The lowest yielding variety was Pioneer 58N57 at 0.60 tons of dry forage per acre. The range between high and low yields was 0.25 and the mean was 0.72. When subjected to analysis of variance there were significant differences among yields by cultivar at this harvest (Table A21).

Yields for each harvest were compiled and then analyzed as total yield for the year of 2007 (Figure 5.2). There were no significant differences among total dry matter yields by cultivar in 2007 (Table A22). Total dry matter yield for 2007 was greatest in AmeriStand 802 at 6.97 tons of dry forage per acre lowest in Pioneer 58N57 at 5.03 tons of dry forage per acre (Table 5.2 and Figure 5.2). The range between high and low yields was 1.94 and the mean was 5.89.

2008 Harvest

In 2008, two issues arose in the plots and caused certain plots to be excluded: water issues on plots in the front of the bay used for this project and root rot development in mid to late summer. As each harvest is discussed below, any issues affecting data will be discussed and the approach to managing the impact on the data analysis.

In the first harvest, conducted April 21, 2008, the highest yielding variety was S&W 9720 at 1.68 tons of dry forage per acre. The lowest yielding variety was tied between Barstow Common and Pioneer 58N57 at 1.39 tons of dry forage per acre. The range between high and low yields was 0.29 and the mean was 1.50. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A23).

At the time of second harvest, May 19, 2008, an issue was apparent in the plots. The six plots in the front replication (101, 102, 103, 104, 105 and 106) were visually shorter in terms of plant height indicating a water issue during irrigation (Figure 5.1). The plots are irrigated from the front to back but it seems as if the water was moving quickly and was not soaking in the front end of the bay. All plots were harvested and there were statistical differences in yield (Table A24). Due to concerns that the affected front six plots were skewing the data (ie, showing irrigation issues and not salt tolerance issues) those six plots were then excluded from the data which were then reanalyzed. Results indicated no significant differences in yield (Table A25). The highest yielding variety was S&W 9720 and FGR105BD101 both at 1.19 tons of dry forage per acre. The lowest yielding variety was tied between Barstow Common at 0.68 tons of dry forage per acre. The range between high and low yields was 0.51 and the mean was 0.99.

In the third harvest, conducted June 13, 2008, the highest yielding variety was S&W 9720 at 1.44 tons of dry forage per acre. The lowest yielding variety was FG91T005GT at 1.39 tons of dry forage per acre. The range between high and low yields was 0.45 and the mean was 1.23. When subjected to analysis of variance there were no significant differences among yields by cultivar at alpha = 0.05 (Table A26). However, given an alpha value = 0.10 this rejects the null hypothesis and indicates statistical differences among yields (Table A27).

At the time of fourth harvest, July 15, 2008, a different issue was apparent in the plots. Six plots in the north-eastern portion of the study were exhibiting varying levels of injury due to root rot. Visual ratings were assigned for percent of injury as follows: 301-35%, 302-30%, 307-5%, 308-80%, 401-60% and 402-10% (Figure 5.1). All plots were harvested and all data were subjected to analysis of variance and no significant differences in yield were apparent (Table A28). Given the p-value and no significant differences being present it was determined that the root rot injury was not skewing the data. The highest yielding variety was S&W 9720 at 1.44 tons of dry forage per acre. The lowest yielding variety was between FG91T005Gt at 0.99 tons of dry forage per acre. The range between high and low yields was 0.45 and the mean was 1.23.

In the fifth harvest, conducted August 11, 2008, the root rot issue had escalated to a level that would have skewed data and subsequently 9 plots were excluded from the data (plots 301, 302, 303, 307, 308, 309, 401, 402, and 403). The highest yielding variety was FGR105BD101 at 1.12 tons of dry forage per acre. The lowest yielding variety was TS0002 at 0.78 tons of dry forage per acre. The range between high and low yields was 0.34 and the mean was 0.99. When subjected to analysis of variance there were significant differences among yields by cultivar at this harvest (Table A29).

In the sixth and final harvest of 2008, conducted September 8, 2008, the same plots that were excluded in the August 11 harvest were not harvested at all due to extreme injury levels. Also hampering this harvest were extremely wet conditions which prevented equipment from being used in the field. Therefore, a 2' x 2' quadrat was used to hand harvest plots using grass shears. The highest yielding variety was Pioneer 58N57 at 1.20 tons of dry forage per acre. The lowest yielding variety was TS8028 at 0.85 tons of dry forage per acre. The range between high and low yields was 0.35 and the mean was 0.97. When subjected to analysis of variance there were no significant differences among yields by cultivar (Table A30).

Yields for each harvest were compiled and then analyzed as total yield for the year of 2008 (Figure 5.3). There were no significant differences among total dry matter yields by cultivar in 2008 at alpha 0.05 (Table A31). However, when analyzed at alpha = 0.10 the null hypothesis was rejected and significant differences were observed (Table A32). Thus, with LSD(0.1) = 0.57 and %CV = 14%, the significantly higher yielding cultivars in 2008 were SW9720, AmeriStand 801S, AmeriStand 802, FGR105BD101 and Salado. Total dry matter yield for 2008 was greatest in SW9720 at 7.60 tons of dry forage per acre lowest in Pioneer TS0002 at 5.78 tons of dry forage per acre. The range between high and low yields was 1.82 and the mean was 6.74.

Conclusion

Summarizing data from the 2007 and 2008 field harvests allows for the observation of trends over the two year period (Table 5.3 and Figure 5.4). Throughout the two-year project significant differences among cultivar yields were observed only 4 out of the 13 (31%) harvests at alpha = 0.05 (6 out 13 exhibited significant differences when using alpha = 0.1). Differences became apparent later in the growing season for both 2007 and 2008 indicating that as flood irrigation with saline water continues salt accumulation and long term stress may have an impact on yields. There were no significant differences when two-year totals were subject to ANOVA (Table A33) or when two-year averages were subject to ANOVA (Table A34). It should be noted that all 12 of the cultivars in this experiment were selected for the ability to tolerate saline conditions based upon breeding or adaptation of the cultivar. Although data were lacking, Barstow Common was the oldest cultivar in the experiment and served as the benchmark for evaluation of the other cultivars based on its ready acceptance by alfalfa producers in the area.

BORDER

101 Pioneer 58N57	**107** CW 59086	**201** FG 91T005GT	**207** FG R105BD101	**301** Barstow	**307** TS 0002	**401** Pioneer 58N57	**407** TS 8028
102 FG R105BD101	**108** Ameris 801S	**202** S&W 9720	**208** TS 8028	**302** CW 39060	**308** Salado	**402** CW 39060	**408** Ameris 802
103 FG 91T005GT	**109** Salado	**203** Ameris 802	**209** TS 0002	**303** Ameris 801S	**309** CW 59086	**403** S&W 9720	**409** FG R105BD101
104 TS 8028	**110** S&W 9720	**204** CW 39060	**210** Barstow	**304** Pioneer 58N57	**310** Ameris 802	**404** FG 91T005GT	**410** CW 59086
105 TS 0002	**111** Ameris 802	**205** Pioneer 58N57	**211** Salado	**305** FG 91T005GT	**311** FG R1058BD101	**405** Ameris 801S	**411** Barstow
106 CW 39060	**112** Barstow	**206** Ameris 801S	**212** CW 59086	**306** S&W 9720	**312** TS 8028	**406** Salado	**412** TS 0002

BORDER

BORDER (left) **BORDER** (right)

Figure 5.1: Field plot map for Alfalfa Production Under Saline Field Conditions Experiment in 2007 and 2008

Table 5.1: Total dry matter yields (tons/acre) of twelve cultivars of alfalfa grown under saline conditions in Pecos, TX with 7 harvest dates in 2007

	2007 Harvest Dates							2007
Variety	**7-May**	**4-Jun**	**2-Jul**	**30-Jul**	**27-Aug**	**24-Sep**	**5-Nov**	**Total**
Ameris802	1.05	0.89	1.16	1.23	1.00^{a}	0.79^{ab}	0.85^{a}	6.97
SW9720	0.93	0.86	1.10	1.16	1.02^{a}	0.85^{a}	0.84^{a}	6.77
FGR105BD101	0.68	0.73	1.03	1.12	1.02^{a}	0.77^{bc}	0.78^{a}	6.15
Salado	0.75	0.75	0.99	1.15	0.95^{ab}	0.78^{ab}	0.76^{a}	6.13
Ameris801S	0.71	0.81	0.89	1.11	1.04^{a}	0.76^{bc}	0.79^{a}	6.11
CW39060	0.63	0.71	0.98	1.05	0.97^{ab}	0.74^{bc}	0.76^{a}	5.83
CW59086	0.62	0.75	0.84	0.98	0.96^{ab}	0.75^{bc}	0.78^{a}	5.69
FG91T005GT	0.71	0.7	0.96	1.02	0.92^{ab}	0.70^{cd}	0.63^{b}	5.64
TS8028	0.80	0.62	0.93	1.04	0.86^{bc}	0.70^{cd}	0.66^{b}	5.61
Barstow	0.68	0.65	0.86	1.03	0.95^{ab}	0.74^{bc}	0.61^{b}	5.51
TS0002	0.73	0.69	0.87	0.92	0.70^{d}	0.65^{d}	0.62^{b}	5.18
Pioneer 58N57	0.69	0.62	0.82	0.89	0.78^{cd}	0.63^{d}	0.60^{b}	5.03
Mean	0.75	0.73	0.95	1.06	0.93	0.74	0.72	5.89
Stand Deviation	0.13	0.09	0.11	0.10	0.10	0.06	0.09	0.58
P-value (0.05)	0.673	0.835	0.665	0.441	0.041	0.025	0.006	0.435
LSD (5%)	NS	NS	NS	NS	0.12	0.07	0.09	NS

LSD (0.05) represents the Least Significant Difference at the 5% level. When the difference between values is equal to or greater than the LSD then they are considered significantly different with 95% confidence.
NS indicates no significant differences exist (alpha = 0.05)
Corresponding letters (a, ab, bc, etc) indicate values with no significant difference.

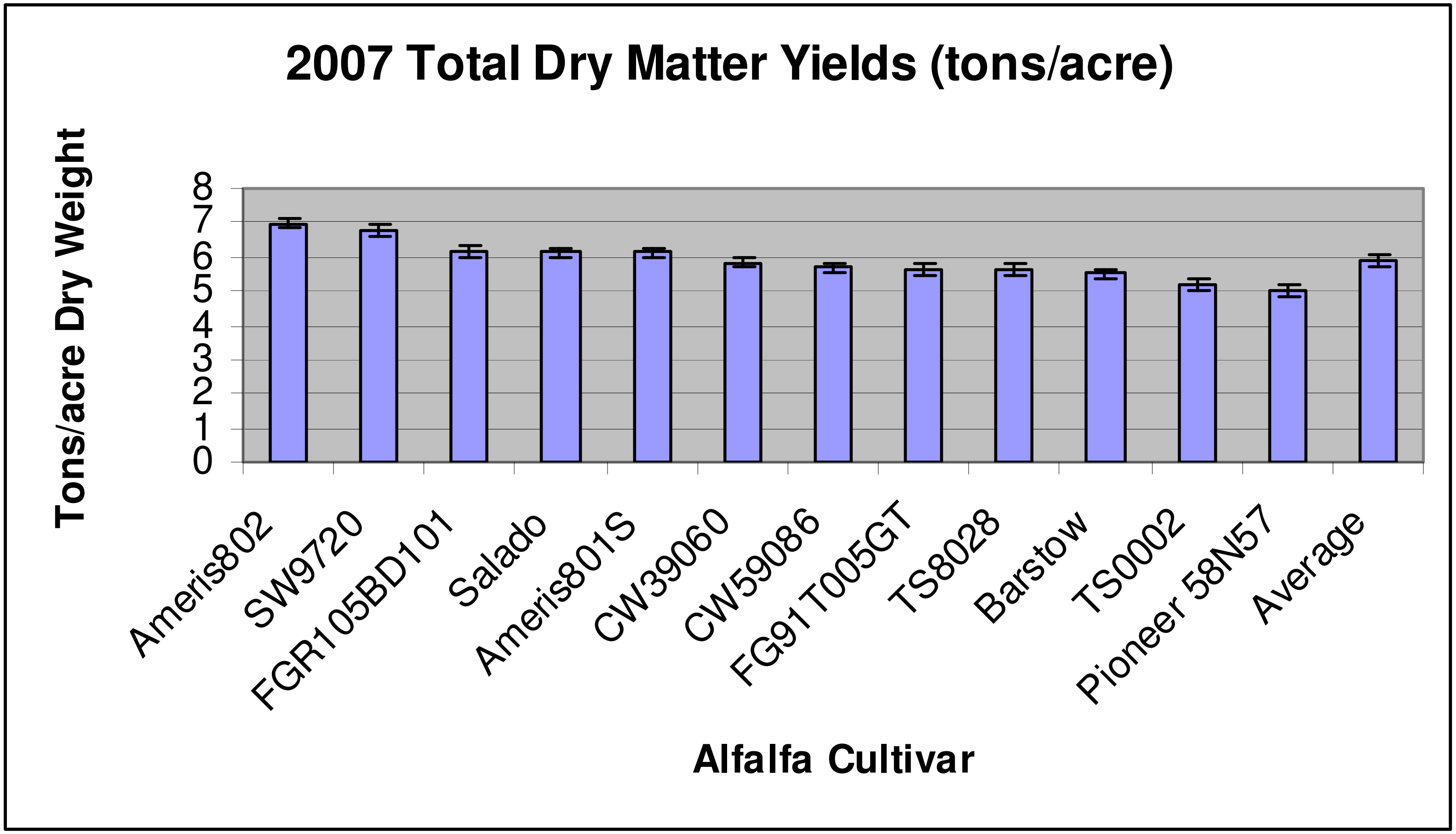

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Figure 5.2: 2007 Total Dry Matter Yields Graph (tons/acre)

Table 5.2: Total dry matter yields (tons/acre) of twelve cultivars of alfalfa grown under saline conditions in Pecos, TX with 6 harvest dates in 2008

	2008 Harvest Dates						2008
Variety	**21-Apr**	**19-May**	**13-Jun**	**15-Jul**	**11-Aug**	**8-Sep**	**Total**
SW9720	1.68	1.20	1.44	1.22	1.04[abc]	1.02	7.60
Ameris801S	1.54	1.20	1.37	1.28	1.08[ab]	1.01	7.49
Ameris802	1.58	1.12	1.31	1.25	1.07[ab]	1.02	7.35
FGR105BD101	1.57	1.08	1.32	1.15	1.12[a]	0.97	7.21
Salado	1.56	1.19	1.37	1.03	0.94[cde]	1.03	7.10
CW59086	1.46	1.04	1.20	1.25	1.00[bcd]	0.96	6.92
CW39060	1.49	0.91	1.29	0.98	1.01[bcd]	0.94	6.62
Pioneer 58N57	1.39	0.77	1.15	0.95	0.92[de]	1.20	6.39
FG91T005GT	1.50	0.81	0.99	0.99	1.04[abc]	0.87	6.21
Barstow	1.39	0.76	1.02	1.03	1.02[abcd]	0.88	6.10
TS8028	1.46	0.88	1.05	0.96	0.86[ef]	0.85	6.06
TS0002	1.42	0.80	1.04	0.86	0.78[f]	0.88	5.78
Mean	1.50	0.98	1.21	1.08	0.99	0.97	6.74
Stand Deviation	0.09	0.18	0.16	0.14	0.10	0.10	0.62
P-value (0.05)	0.543	0.071	0.089	0.111	0.041	0.611	0.066
LSD (5%)	NS	NS	NS	NS	0.10	NS	NS

LSD (0.05) represents the Least Significant Difference at the 5% level. When the difference between values is equal to or greater than the LSD then they are considered significantly different with 95% confidence.
NS indicates no significant differences exist (alpha = 0.05)
Corresponding letters (a, ab, bc, etc) indicate values with no significant difference.
Note: Plots were removed from Cut 2 due to visual appearance of water issues; Plots were removed from Cut 3 due to root rot injury and all subsequent harvests

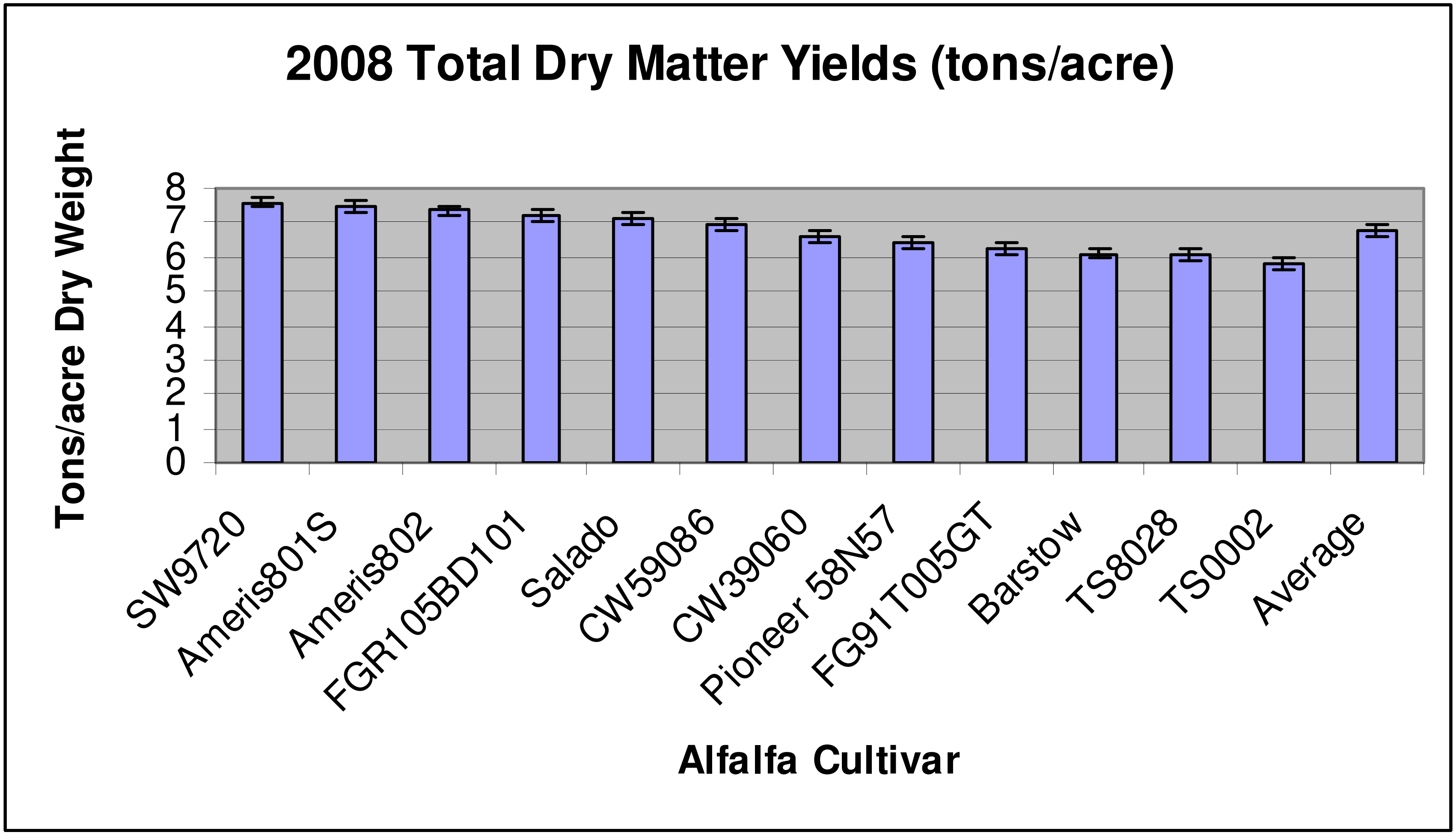

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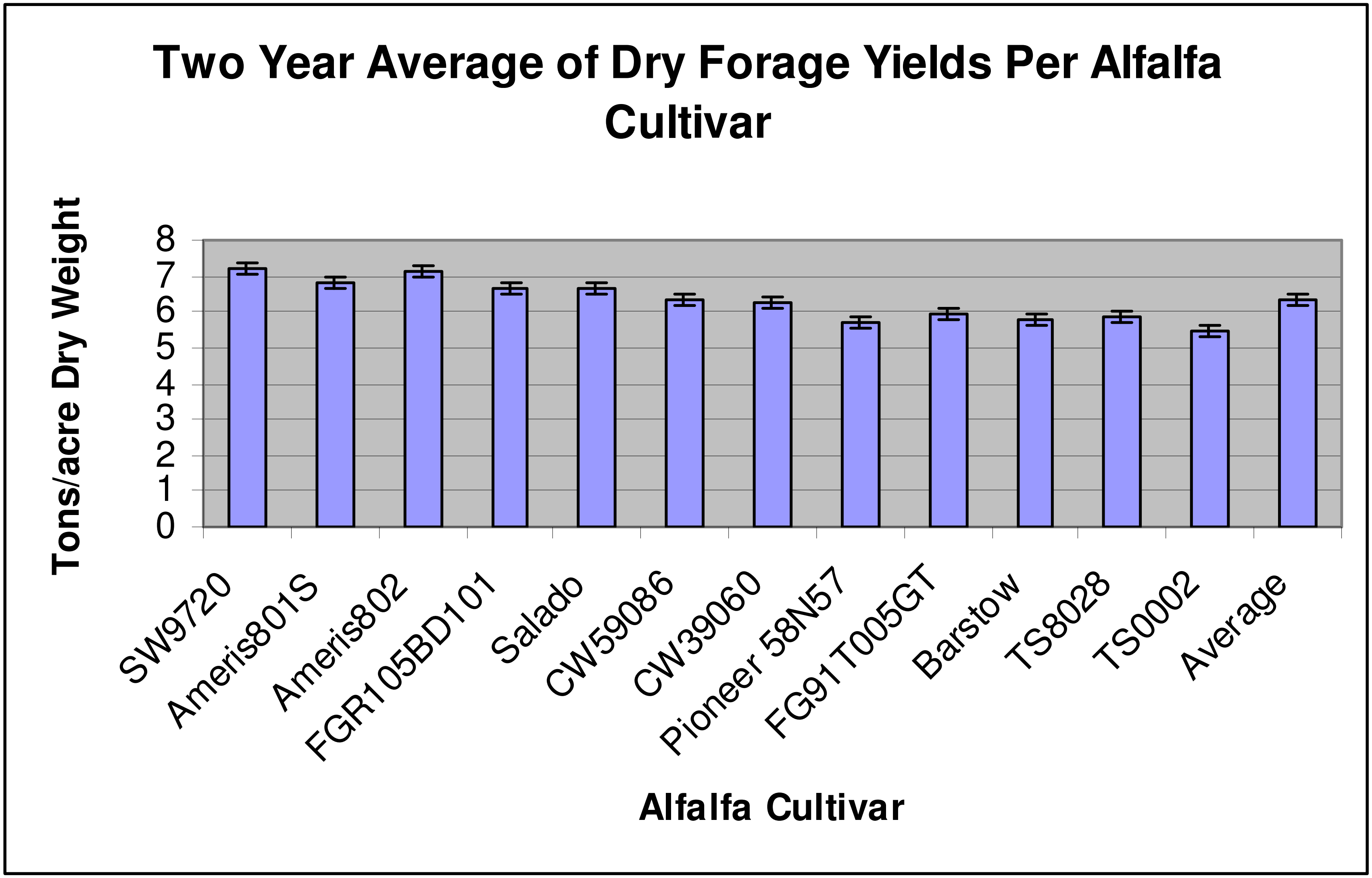
Table Figure 5.3: 2008 Total Dry Matter Yields Graph (tons/acre)

5.3: Two year data summary of dry matter yields (tons/acre) for 2007 and 2008 field harvests

	2007 Total	2008 Total	2 Year Total	2 Year Average
SW9720	6.77	7.60*	14.37*	7.19*
Ameris802	6.97*	7.35	14.32	7.16
Ameris801S	6.11	7.49	13.60	6.80
FGR105BD101	6.15	7.21	13.36	6.68
Salado	6.13	7.10	13.23	6.62
CW59086	5.69	6.92	12.61	6.31
CW39060	5.83	6.62	12.45	6.23
FG91T005GT	5.64	6.21	11.85	5.93
TS8028	5.61	6.06	11.67	5.84
Barstow	5.51	6.10	11.61	5.81
Pioneer 58N57	5.03	6.39	11.42	5.71
TS0002	5.18	5.78	10.96	5.48
Mean	5.89	6.74	12.62	6.31
Stand Deviation	0.58	0.62	1.58	0.58
LSD (5%)	NS	NS	NS	NS

LSD (0.05) represents the Least Significant Difference at the 5% level. When the difference between values is equal to or greater than the LSD then they are considered significantly different with 95% confidence.
*Highest numerical value in the column
NS - Not significantly different from the highest numerical value in the column based on the 5% LSD



Error bars equal standard error

Figure 5.4: Bar graph of two year data summary of dry matter yields (tons/acre) for 2007 and 2008 field harvests

CHAPTER VI
ANALYSIS OF FORAGE QUALITY DIFFERENCES BETWEEN FALL DORMANCY RATINGS OF ALFALFA

Introduction

Fall dormancy (FD) ratings estimate winter growth and photosynthetic activity, and are commonly used as a guide to select alfalfa cultivars adapted to fall and winter conditions specific to a growing region. The FD rating scale ranges from 10 to 1 with FD 10 cultivars being non-dormant and ideal for the southern and southwestern US and FD1 being extremely winter dormant and ideal for the north and northeastern US. This project utilized forage samples taken in Experiment 3: *Alfalfa Production Under Saline Field Conditions* to develop data to analyze forage quality differences between fall dormancy ratings. Recent research has indicated that fall dormancy of the variety was an important predictor of quality and that more dormant varieties were almost always higher in quality. An average change of 0.62% ADF per FD unit was observed and similar changes in NDF and CP (Putnam et al., 2005). Ideally, these data will be useful to hay growers producing high quality alfalfa hay for dairy or equine operations and assist in selection of cultivars with FD ratings that will produce the highest quality forage possible if indeed significant differences do exist.

Materials and Methods

Samples were collected during the 2007 growing season and submitted to the University of Wisconsin – Soil and Forage Testing Laboratory in Marshfield, Wisconsin for analysis. The two cultivars analyzed were Pioneer 58N57 (FD – 8) and Forage Genetics R105BD101 (FD – 10). These cultivars were selected at random from the cultivars grown in the field experiment for the sole purpose of having a FD 8 and FD 10 cultivar to evaluate. Samples were taken at 6 harvests: May 7, 2007 (Cut 1), June 4, 2007 (Cut 2), July 2, 2007 (Cut 3), August 27, 2007 (Cut 5), September 24, 2007 (Cut 6) and November 5, 2007 (Cut 7). Samples were air dried, stored in sealed plastic bags and then ground prior to shipment (all samples were sent at the same time). Samples were obtained from the randomized complete block design of the field plots and thus this experiment was replicated 4 times per variety per cut.

Forage quality parameters analyzed included: Crude Protein (CP), Rumen Degraded Protein (RDP), Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), Total Digestible Nutrient (TDN) and Relative Forage Quality (RFQ). Information regarding the various forage quality parameters analyzed was gathered from various sources, including the University of Wisconsin Extension publication "Understanding Forage Quality" (Ball et al, 2001). Data were subjected to analysis of variance to assess statistical differences in forage quality (Table 6.1).

<u>Results</u>

The average crude protein (CP) value across all samples from the entire growing season was 22.35% indicating a very high quality and nutritive forage (Figure 6.1). Crude protein values were numerically very similar over the course of the growing season. Data were analyzed based on averages for each cutting to evaluate differences of crude protein values between cultivars over time (analysis included replications). There were no significant differences in crude protein values between cultivars (Table A35).

The average acid detergent fiber (ADF) across all samples from the entire growing season was 27.74 indicating very palatable and digestible forage. Data were analyzed based on averages for each cutting to evaluate differences in acid detergent fiber between cultivars over time (analysis included replications). There were no significant differences in ADF values between cultivars (Table A36).

The average neutral detergent fiber (NDF) value across all samples from the entire growing season was 33.55 indicating digestibility of the samples (indigestible or slowly digestible plant parts). Data were analyzed based on averages for each cutting to evaluate differences in acid detergent fiber between cultivars over time (analysis included replications). . There were no significant differences in NDF values between cultivars (Table A37).

The average rumen degraded protein (RDP) value across all samples from the entire growing season was 82.43 indicating the amount of total protein degraded to ammonia in the rumen. Data were analyzed based on averages for each cutting to evaluate differences in acid detergent fiber between cultivars over time (analysis included replications). . There were no significant differences in RDP values between cultivars (A38).

The average total digestible nutrients (TDN) value across all samples from the entire growing season was 60.18 indicating forage that would effectively meet energy demands of all classes of livestock (especially the high energy needs of dairy cattle or lactating beef cows). Data were analyzed based on averages for each cutting to evaluate differences in acid detergent fiber between cultivars over time (analysis included replications). There were no significant differences in total digestible nutrients values were between cultivars (Table A39).

The average relative forage quality (RFQ) value across all samples from the entire growing season was 173.93, indicating high quality forage that will optimize animal performance. Data were analyzed based on averages for each cutting to evaluate differences in acid detergent fiber between cultivars over time (analysis included replications). There were no significant differences in relative feed quality were between cultivars (Table A40).

Data were then analyzed at each cutting for each parameter and showed no significant differences between cultivars at each individual cutting except for the TDN and RFQ values for the August 27, 2007 harvest (Table 6.2). The TDN values when analyzed (alpha = 0.05) indicated significant differences between the TDN values between cultivars at this harvest (Table A40). Average TDN for Pioneer 58N57 (FD8) = 60.87 versus TDN for Forage Genetics FGR105BD101 (FD10) = 57.59. The RFQ values when analyzed (alpha = 0.1) indicated significant differences between the RFQ values between cultivars at this harvest (Table A41). Average RFQ for Pioneer 58N57 (FD8) = 170.53 versus RFQ for Forage Genetics FGR105BD101 (FD10) = 144.98.

<u>Conclusion</u>

The data show that there were no significant differences between the FD8 and FD10 alfalfa cultivars evaluated over the course of a growing season. The data does, however, show that differences can exist at individual cuttings during the growing season although it seems to be more the exception than the rule. Of the six cuttings and six forage quality parameters analyzed in this experiment significant differences were found to occur only at one cutting for only 2 forage quality parameters. It should be noted that TDN values are calculated based upon crude protein and acid detergent fiber which were not statistically different when analyzed by cut or by yearly averages. RFQ is calculated using 6 other parameters and is based largely on TDN and intake potential (Ball et al,

2001) so it would be expected that variation seen in TDN values would also be present when analyzing RFQ values. Therefore, forage quality differences between FD8 and FD10 alfalfa cultivars may occur but in general should not be a factor for producers when selecting cultivars. The primary factor relating to FD ratings should be suitability for the growing region.

Table 6.1: Average forage quality values FD8 and FD10 cultivars over the growing season

Pioneer 85N57

Harvest Date	CP	ADF	NDF	RDP	TDN	RFQ
May 7, 2007	21.56	29.04	34.34	82.89	55.95	150.24
Jun. 4, 2007	20.79	28.98	36.51	82.11	59.05	155.29
Jul. 2, 2007	22.73	28.45	34.63	83.55	60.50	171.50
Aug. 27, 2007	20.76	27.89	33.83	81.21	60.87	170.53
Sep. 24, 2007	24.20	26.98	31.70	82.61	62.91	199.35
Nov. 5, 2007	23.97	25.04	30.47	82.99	61.62	200.37
AVERAGE	**22.33**	**27.73**	**33.58**	**82.56**	**60.15**	**174.55**

Forage Genetics FGR105BD101

Harvest Date	CP	ADF	NDF	RDP	TDN	RFQ
May 7, 2007	21.38	28.36	33.09	83.84	58.06	166.96
Jun. 4, 2007	20.98	27.16	34.01	83.18	60.77	169.51
Jul. 2, 2007	22.49	28.32	34.95	82.81	60.14	165.11
Aug. 27, 2007	20.48	29.78	36.39	79.67	57.59	144.98
Sep. 24, 2007	24.59	27.36	32.60	81.47	61.77	186.33
Nov. 5, 2007	24.27	25.50	30.09	82.83	62.90	207.00
AVERAGE	**22.36**	**27.75**	**33.52**	**82.30**	**60.21**	**173.32**
Average btwn cutivars	**22.35**	**27.74**	**33.55**	**82.43**	**60.18**	**173.93**

Table 6.2: P-values of FD8 and FD10 alfalfa cultivars for forage quality analysis parameters

Harvest Date	CP	ADF	NDF	RDP	TDN	RFQ
May 7, 2007	0.749	0.630	0.348	0.633	0.294	0.191
Jun. 4, 2007	0.808	0.457	0.413	0.470	0.469	0.480
Jul. 2, 2007	0.737	0.951	0.905	0.626	0.870	0.749
Aug. 27, 2007	0.191	0.198	0.257	0.423	0.024*	0.067**
Sep. 24, 2007	0.690	0.759	0.704	0.418	0.598	0.570
Nov. 5, 2007	0.690	0.696	0.817	0.814	0.244	0.660
AVERAGE	0.975	0.984	0.963	0.715	0.966	0.922

* indicates significant difference (alpha = 0.05)
** indicates significant difference (alpha = 0.1)

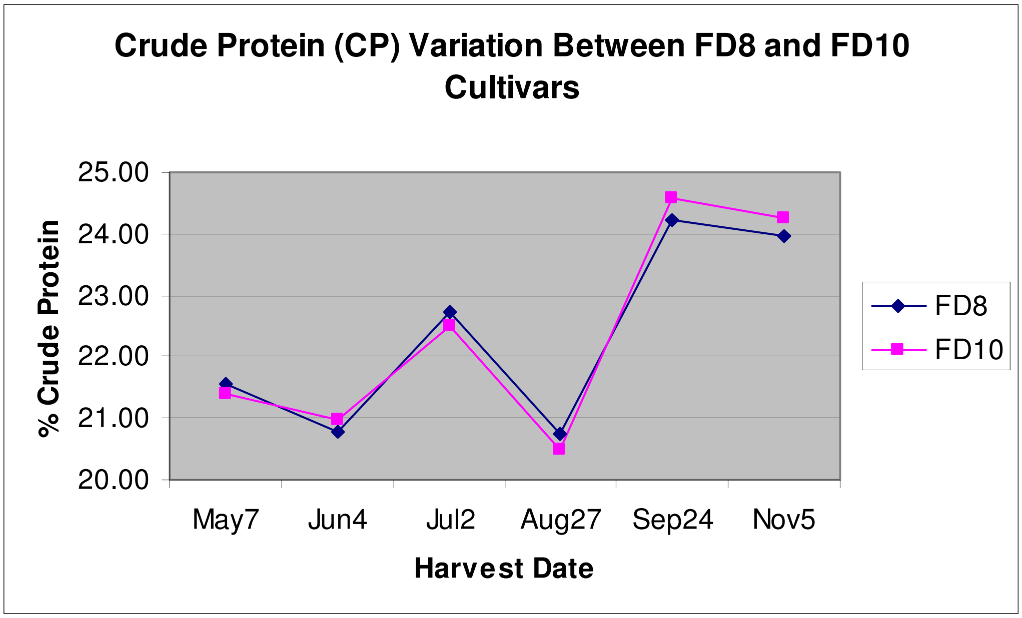


Figure 6.1: Line graph comparison of Crude Protein (CP) values between FD8 and FD10 cultivars over the 2007 growing season

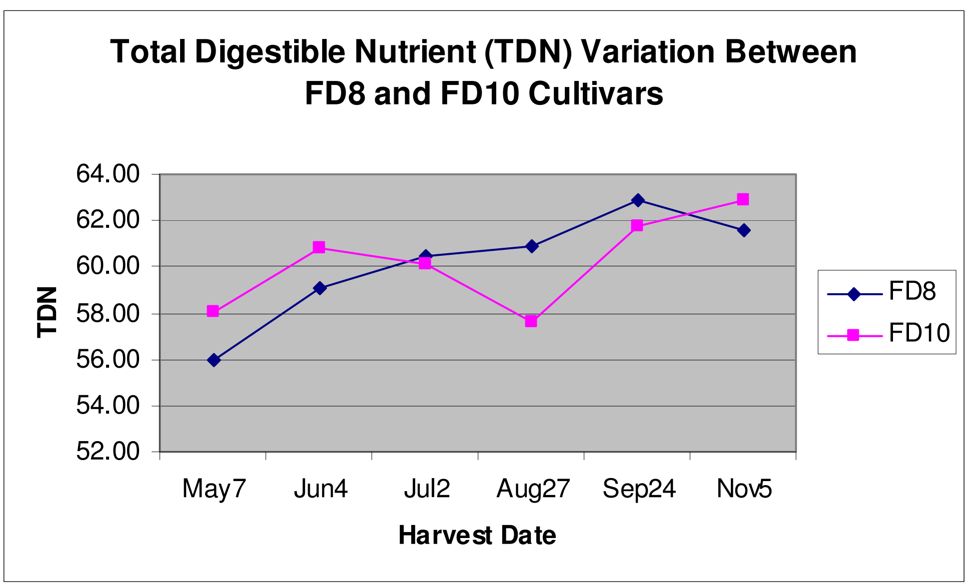


Figure 6.2: Line graph comparison of Total Digestible Nutrient (TDN) values between FD8 and FD10 cultivars over the 2007 growing season

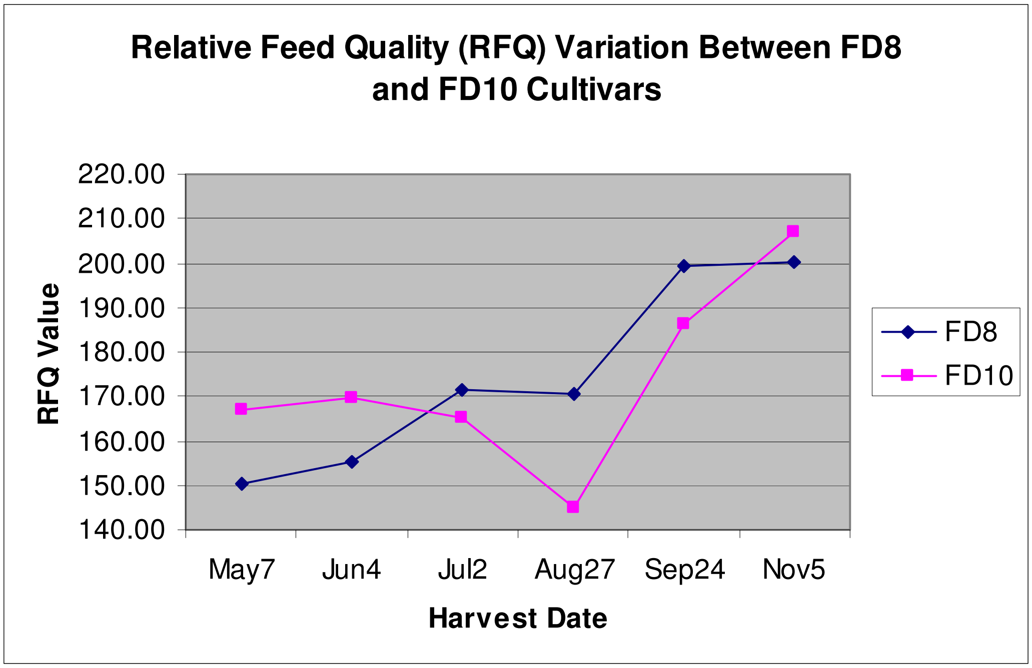


Figure 6.3: Line graph comparison of Relative Feed Quality (RFQ) values between FD8 and FD10 cultivars over the 2007 growing season

CHAPTER VII
COMPARISON OF EXPERIMENTS AND CONCLUSIONS

In order to analyze salt tolerance in alfalfa for this project it is necessary to compare the following experiments: Salt Tolerance of Germinating Alfalfa Seeds (GERM), Forage Production of Alfalfa Under Salt Stress (PROD) and Alfalfa Production Under Saline Field Conditions (FIELD).

In the GERM experiment, statistical differences were observed for percent germination among cultivars which would indicate a variance in stand establishment under saline conditions. Problems at establishment will turn into long term production issues, so it is important for a cultivar to have salt tolerance at the germination stage to ensure good stand establishment and to have the potential to optimize long term yields. These differences in turn are exhibited in the IC(50) values which reflect osmotic potential required to inhibit 50% of seed from germination.

In the PROD experiment, statistical differences of SCR values did not occur at any of the three cuts among cultivars (although numerical differences were observed) but statistical differences did occur between cuts. This indicates that among these cultivars no differences exist in terms of regrowth potential under salt stress but the differences between cuts does indicate that differences can occur over time.

In the FIELD experiment, statistical differences of forage yields were seen only in a few harvests (Harvest #7 – 2007 and Harvest #2 – 2008) and were generally the exception rather than the rule. However, the differences seemed to become more apparent in the long term and perhaps salt stress has a more severe impact over the long term (ie, salt loading, etc).

When comparing GERM versus PROD a linear relationship exists when percent germination values are graphed with SCR values (Figure 7.1 – 7.4). As the salt solution increases the relationship tends to decrease. At 0.5%, 1.0% and 1.5% the relationship is neutral while at 2.0% the relationship is slightly negative indicating that as percent germination increases regrowth potential decreases (R Square for 2.0% comparison = 0.015) (Table A43 – A46). However, when GERM is compared to Average Saline Yields (grams) a positive relationship is expressed indicating that as percent germination increases potential yield under saline conditions increases (Figure 7.2).

When comparing FIELD versus GERM a linear relationship exists when the germination values at a 2% solution are graphed with total yields for two years (R Square = 0.248) (Figure 7.3 and Table A44). The relationship is positive indicating that as percent germination increases the potential field production and yield also increases.

When comparing FIELD versus PROD a linear relationship exists when SCR values are graphed with total 2 year yields (Figure 7.4). The relationship is slightly negative indicating that as SCR values increase production potential in the field decreases (R Square = 0.005) (Figure 7.4 and Table A45). However, when FIELD is compared to average yields under the saline treatment a positive relationship is expressed indicating that as average saline yields in the greenhouse increases, potential yield under saline conditions in the field increase (Figure 7.5).

Twelve alfalfa cultivars were carried through three primary salt screening procedures. Each cultivar was ranked in each experiment and then a cumulative value was calculated and a ranking was assigned to each cultivar. That value was then subtracted from 100 to create a score used to put a final ranking to the varieties for comparison purposes. The top ranking cultivars were Salado, S&W 9720, TS8028, AmeriStand 802, FGR105BD101, CW59086, AmeriStand 801S, Pioneer 58N57, Barstow Common, CW39060, FG91T005GT, TS0002 (Table 7.1).

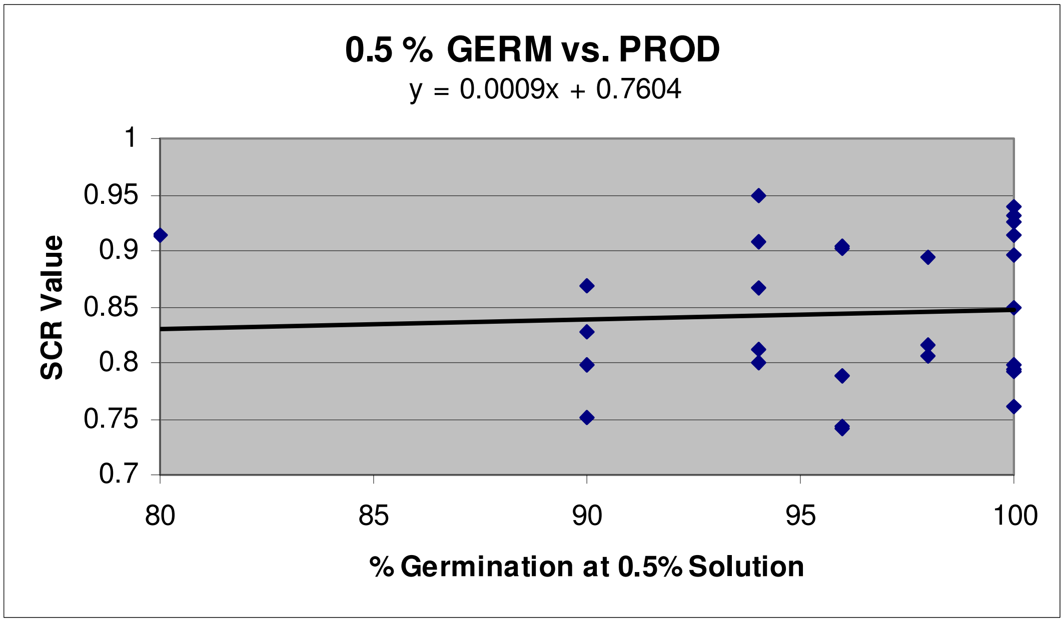


Figure 7.1: Linear Regression of GERM versus PROD (Germination Rates at 0.50% solution versus SCR Values)

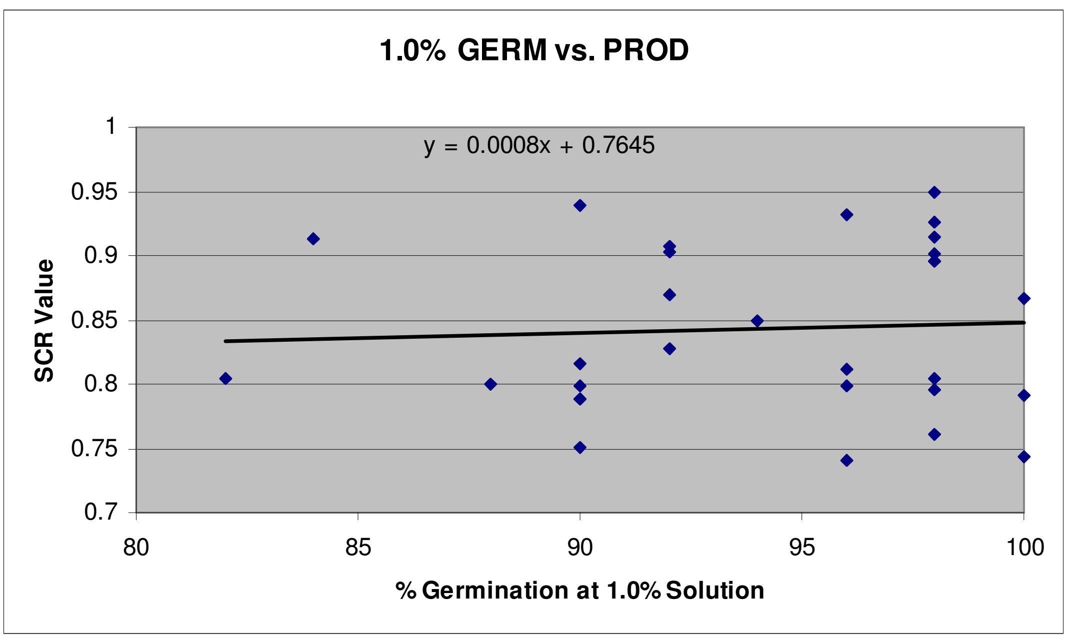


Figure 7.2: Linear Regression of GERM versus PROD (Germination Rates at 1.00% solution versus SCR Values)

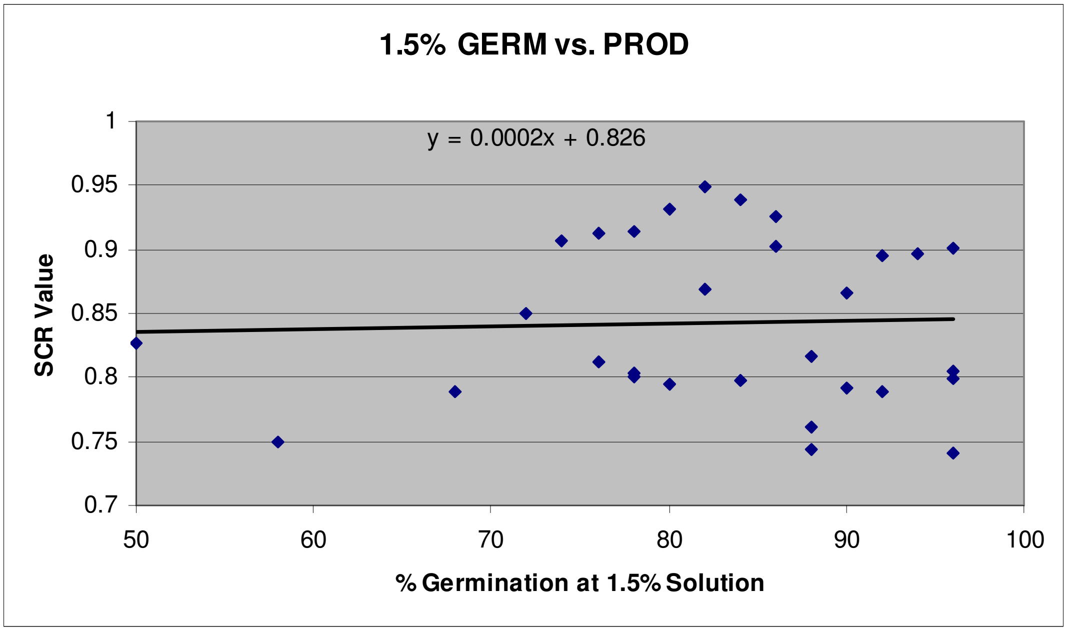


Figure 7.3: Linear Regression of GERM versus PROD (Germination Rates at 1.50% solution versus SCR Values)

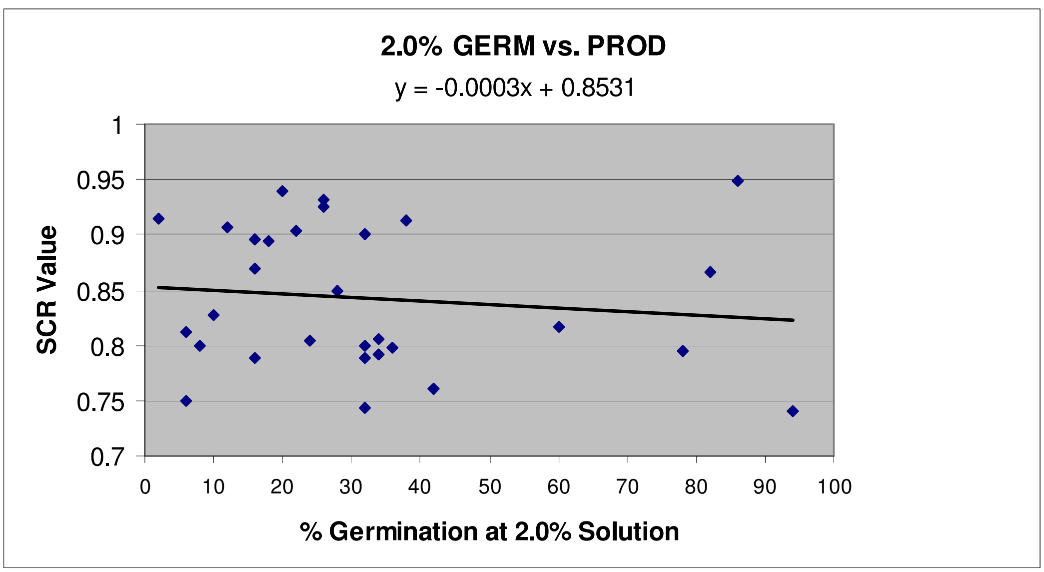


Figure 7.4: Linear Regression of GERM versus PROD (Germination Rates at 2% solution versus SCR Values)

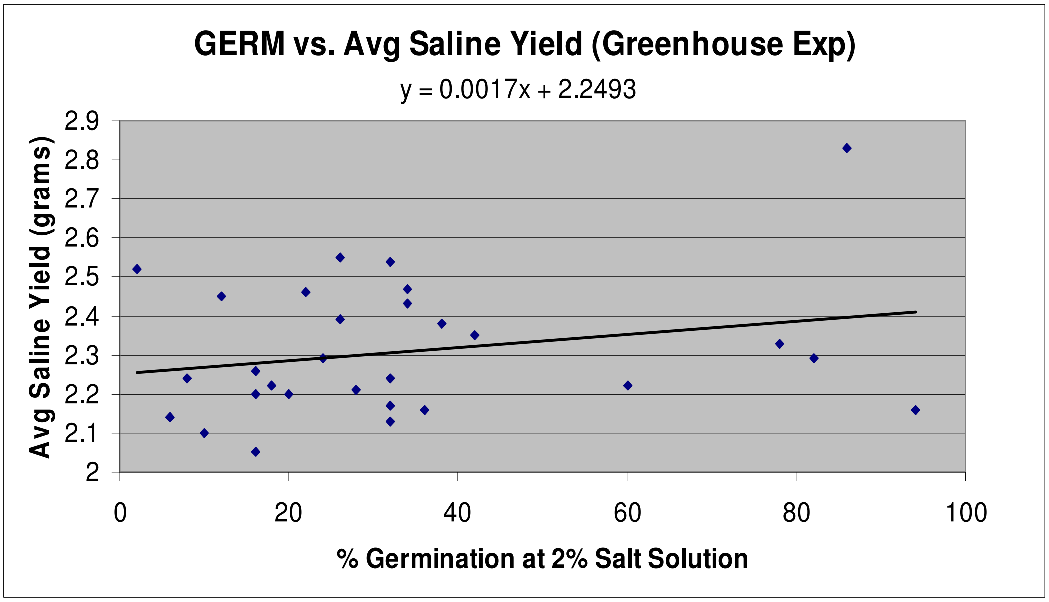


Figure 7.5: Linear Regression of GERM versus PROD (Germination Rates at 2% solution versus average yields under saline irrigation)

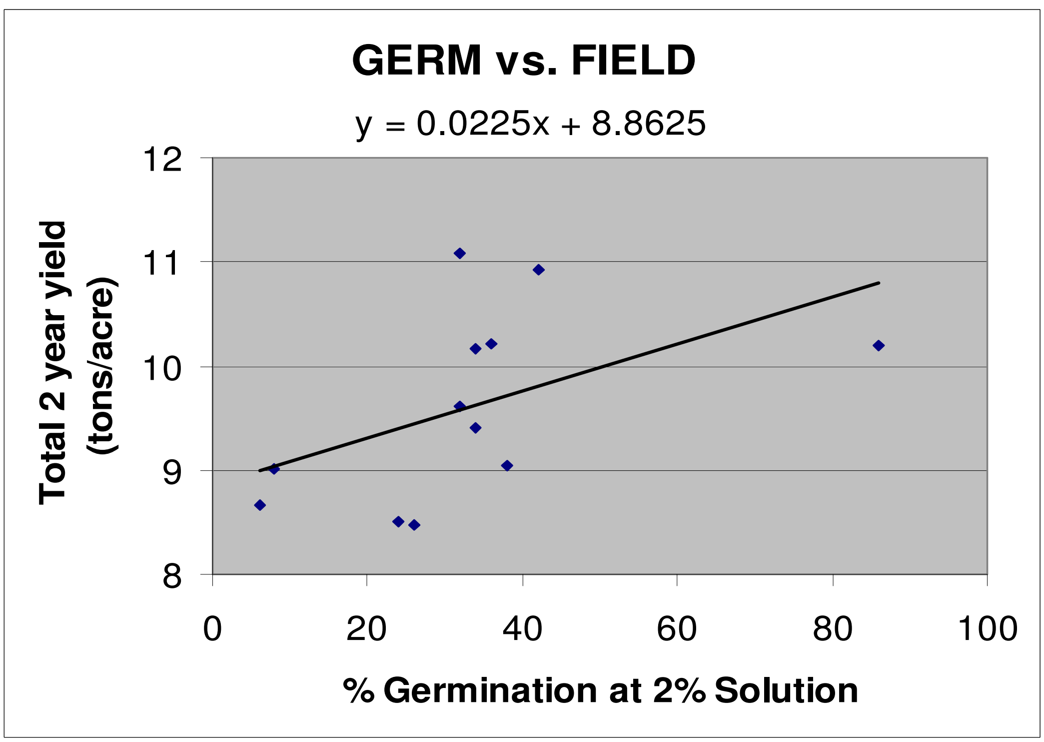


Figure 7.6: Linear regression of GERM versus FIELD (Germination Rates at 2% solution versus average two year total yields)

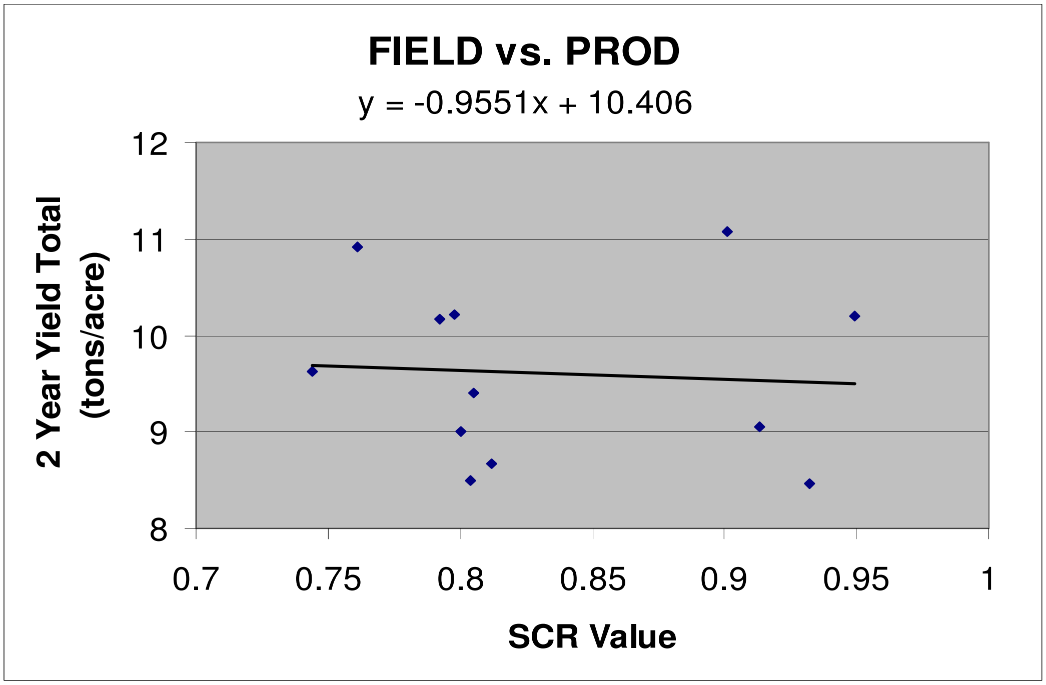


Figure 7.7 Linear regression of FIELD versus PROD (SCR values versus average two year total yields)

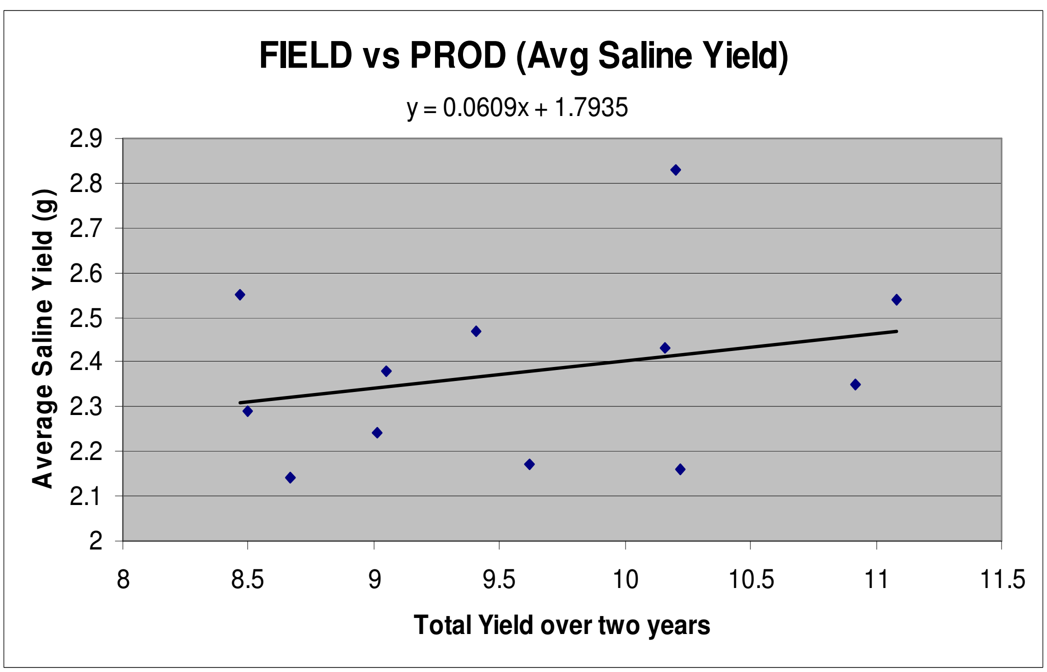


Figure 7.8 Linear regression of FIELD versus PROD (average saline yield values versus average two year total yields)

Table 7.1: Rankings of alfalfa cultivars based on cumulative performance in three screening trials

Rank	Variety	SCORE
1	Salado	93
2	SW9720	88
3	TS8028	86
4	Ameris802	85
5	FGR105BD101	83
6	CW59086	82
7	Ameris801S	82
8	Pioneer 58N57	78
9	Barstow	73
10	CW39060	73
11	FG91T005GT	72
12	TS0002	71

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APPENDIX A
ANALYSES OF VARIANCE

Table A1: Analysis of variance for the mean percent germination of alfalfa seeds under the 0.00% salt concentration in the "Salt Tolerance of Germinating Alfalfa Seeds" experiment

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1415.529	33	42.89483	1.111604	0.379948	1.777407
Within Groups	1312	34	38.58824			
Total	2727.529	67				

Table A2: Analysis of variance for the mean percent germination of alfalfa seeds under the 0.50% salt concentration in the "Salt Tolerance of Germinating Alfalfa Seeds" experiment

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3719.765	33	112.7201	2.207653	0.01216	1.777407
Within Groups	1736	34	51.05882			
Total	5455.765	67				

Table A3: Analysis of variance for the mean percent germination of alfalfa seeds under the 1.00% salt concentration in the "Salt Tolerance of Germinating Alfalfa Seeds" experiment

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11795.06	33	357.426	4.617205	1.22E-05	1.777407
Within Groups	2632	34	77.41176			
Total	14427.06	67				

Table A4: Analysis of variance for the mean percent germination of alfalfa seeds under the 1.50% salt concentration in the "Salt Tolerance of Germinating Alfalfa Seeds" experiment

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19612.24	33	594.3102	4.857343	6.81E-06	1.777407
Within Groups	4160	34	122.3529			
Total	23772.24	67				

Table A5: Analysis of variance for the mean percent germination of alfalfa seeds under the 2.00% salt concentration in the "Salt Tolerance of Germinating Alfalfa Seeds" experiment

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	38446.12	33	1165.034	7.013306	7.49E-08	1.777407
Within Groups	5648	34	166.1176			
Total	44094.12	67				

Table A6: Analysis of variance for the mean Salt/Control Ratio (SCR) values for Cut #2 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.158484	31	0.005112	0.807722	0.721242	1.834694
Within Groups	0.189881	30	0.006329			
Total	0.348365	61				

Table A7: Analysis of variance for the arctransformed mean Salt/Control Ratio (SCR) values for Cut #2 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.291515	31	0.009404	0.681461	0.850336	1.862606
Within Groups	0.386381	28	0.013799			
Total	0.677896	59				

Table A8: Analysis of variance for the mean Salt/Control Ratio (SCR) values for Cut #3 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.576596	31	0.0186	0.393319	0.994125	1.834694
Within Groups	1.418684	30	0.047289			
Total	1.995279	61				

Table A9: Analysis of variance for the arctransformed mean Salt/Control Ratio (SCR) values for Cut #3 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.475477	31	0.015338	0.242555	0.999278	2.461136
Within Groups	0.758821	12	0.063235			
Total	1.234299	43				

Table A10: Analysis of variance for the mean Salt/Control Ratio (SCR) values for Cut #4 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.478029	31	0.01542	1.170463	0.334095	1.834694
Within Groups	0.395236	30	0.013175			
Total	0.873264	61				

Table A11: Analysis of variance for the arctransformed mean Salt/Control Ratio (SCR) values for Cut #4 in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.964091	31	0.0311	0.53727	0.953802	1.848152
Within Groups	1.678656	29	0.057885			
Total	2.642746	60				

Table A12: Analysis of variance for the mean Salt/Control Ratio (SCR) values over the three harvests in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.466706	31	0.015055	3.113142	6.2E-05	1.630387
Within Groups	0.309502	64	0.004836			
Total	0.776208	95				

Table A13: Analysis of variance for the arctransformed mean Salt/Control Ratio (SCR) values over the three harvests in the "Forage Production Under Salt Stress Experiment"

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.772769	31	0.024928	2.766359	0.000384	1.645532
Within Groups	0.531657	59	0.009011			
Total	1.304427	90				

Table A14: Analysis of variance for field plot soil salinity values in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Salinity Values

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	38820622.688	1	38820622.688	142.695	.001
	Error	816161.396	3	272053.799(a)		
Cultivar	Hypothesis	661617.563	11	60147.051	.626	.793
	Error	3168569.354	33	96017.253(b)		
Block	Hypothesis	816161.396	3	272053.799	2.833	.053
	Error	3168569.354	33	96017.253(b)		
Cultivar * Block	Hypothesis	3168569.354	33	96017.253	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =35%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Salinity	48	64.00	1537.00	899.3125	314.41768	98858.475
Valid N (listwise)	48					

Table A15: Analysis of variance for May 7, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut1 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	26.891	1	26.891	51.212	.006
	Error	1.575	3	.525(a)		
Cultivar	Hypothesis	.706	11	.064	.762	.673
	Error	2.777	33	.084(b)		
Block	Hypothesis	1.575	3	.525	6.240	.002
	Error	2.777	33	.084(b)		
Cultivar * Block	Hypothesis	2.777	33	.084	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =44%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut1	48	.38	1.96	.7485	.32805	.108
Valid N (listwise)	48					

Table A16: Analysis of variance for June 4, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut2 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	25.736	1	25.736	270.605	.000
	Error	.285	3	.095(a)		
Cultivar	Hypothesis	.332	11	.030	.574	.835
	Error	1.735	33	.053(b)		
Block	Hypothesis	.285	3	.095	1.809	.165
	Error	1.735	33	.053(b)		
Cultivar * Block	Hypothesis	1.735	33	.053	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =31%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut2	48	.27	1.20	.7322	.22371	.050
Valid N (listwise)	48					

Table A17: Analysis of variance for July 2, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut3 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	43.618	1	43.618	130.514	.001
	Error	1.003	3	.334(a)		
Cultivar	Hypothesis	.492	11	.045	.772	.665
	Error	1.911	33	.058(b)		
Block	Hypothesis	1.003	3	.334	5.771	.003
	Error	1.911	33	.058(b)		
Cultivar * Block	Hypothesis	1.911	33	.058	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =28%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut3	48	.53	1.69	.9533	.26917	.072
Valid N (listwise)	48					

Table A18: Analysis of variance for July 30, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut4 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	53.730	1	53.730	157.695	.001
	Error	1.022	3	.341(a)		
Cultivar	Hypothesis	.436	11	.040	1.033	.441
	Error	1.265	33	.038(b)		
Block	Hypothesis	1.022	3	.341	8.887	.000
	Error	1.265	33	.038(b)		
Cultivar * Block	Hypothesis	1.265	33	.038	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =23%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut4	48	.69	1.56	1.0580	.24070	.058
Valid N (listwise)	48					

Table A19: Analysis of variance for August 27, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut5 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	41.666	1	41.666	240.529	.001
	Error	.520	3	.173(a)		
Cultivar	Hypothesis	.468	11	.043	2.179	.041
	Error	.644	33	.020(b)		
Block	Hypothesis	.520	3	.173	8.877	.000
	Error	.644	33	.020(b)		
Cultivar * Block	Hypothesis	.644	33	.020	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =20%
LSD (0.05) = 0.118

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut5	48	.50	1.26	.9317	.18631	.035
Valid N (listwise)	48					

Table A20: Analysis of variance for September 24, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut6 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	26.113	1	26.113	309.853	.000
	Error	.253	3	.084(a)		
Cultivar	Hypothesis	.168	11	.015	2.409	.025
	Error	.209	33	.006(b)		
Block	Hypothesis	.253	3	.084	13.285	.000
	Error	.209	33	.006(b)		
Cultivar * Block	Hypothesis	.209	33	.006	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =16%
LSD (0.05) = 0.065

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut6	48	.49	.97	.7376	.11580	.013
Valid N (listwise)	48					

Table A21: Analysis of variance for November 5, 2007 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut7 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	25.108	1	25.108	405.270	.000
	Error	.186	3	.062(a)		
Cultivar	Hypothesis	.376	11	.034	3.122	.006
	Error	.361	33	.011(b)		
Block	Hypothesis	.186	3	.062	5.664	.003
	Error	.361	33	.011(b)		
Cultivar * Block	Hypothesis	.361	33	.011	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =19%
LSD (0.05) = 0.087

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut7	48	.46	1.04	.7232	.14009	.020
Valid N (listwise)	48					

Table A22: Analysis of variance for the 2007 field harvest total yield in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Total 2007 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	1662.115	1	1662.115	173.588	.001
	Error	28.725	3	9.575(a)		
Cultivar	Hypothesis	14.698	11	1.336	1.040	.435
	Error	42.380	33	1.284(b)		
Block	Hypothesis	28.725	3	9.575	7.456	.001
	Error	42.380	33	1.284(b)		
Cultivar * Block	Hypothesis	42.380	33	1.284	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =23%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Total 2007 Yield	48	3.59	9.60	5.8845	1.35115	1.826
Valid N (listwise)	48					

Table A23: Analysis of variance for the April 21, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut1 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	108.567	1	108.567	913.710	.000
	Error	.356	3	.119(a)		
Cultivar	Hypothesis	.320	11	.029	.909	.543
	Error	1.055	33	.032(b)		
Block	Hypothesis	.356	3	.119	3.716	.021
	Error	1.055	33	.032(b)		
Cultivar * Block	Hypothesis	1.055	33	.032	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =23%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut1	48	1.14	1.95	1.5039	.19193	.037
Valid N (listwise)	48					

Table A24: Analysis of variance for the May 19, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" with the affected 6 front plots included for analysis

Tests of Between-Subjects Effects - Dependent Variable: Cut2 Yield (w/ front plots)

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	46.179	1	46.179	2564.150	.000
	Error	.054	3	.018(a)		
Cultivar	Hypothesis	1.365	11	.124	2.471	.022
	Error	1.658	33	.050(b)		
Block	Hypothesis	.054	3	.018	.359	.783
	Error	1.658	33	.050(b)		
Cultivar * Block	Hypothesis	1.658	33	.050	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)

Table A25: Analysis of variance for the May 19, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" without the affected 6 front plots included for analysis

Tests of Between-Subjects Effects - Dependent Variable: Cut2 Yield (w/o front plots)

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	41.225	1	41.225	626.133	.000
	Error	.209	3.175	.066(a)		
Cultivar	Hypothesis	.878	11	.080	1.994	.071
	Error	1.081	27	.040(b)		
Block	Hypothesis	.201	3	.067	1.676	.196
	Error	1.081	27	.040(b)		
Cultivar * Block	Hypothesis	1.081	27	.040	.	.
	Error	.000	0	.(c)		

a .954 MS(Block) + .046 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =23%
LSD (0.05) – Not Significant
LSD (0.1) = 0.139

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut2without	42	.62	1.48	1.0266	.23596	.056
Valid N (listwise)	42					

Table A26: Analysis of variance for the June 13, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" (alpha 0.05)

Tests of Between-Subjects Effects - Dependent Variable: Cut3 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	70.737	1	70.737	2310.982	.000
	Error	.092	3	.031(a)		
Cultivar	Hypothesis	1.101	11	.100	1.829	.089
	Error	1.805	33	.055(b)		
Block	Hypothesis	.092	3	.031	.560	.645
	Error	1.805	33	.055(b)		
Cultivar * Block	Hypothesis	1.805	33	.055	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)

Table A27: Analysis of variance for the June 13, 2008 field harvest in the "Forage Production Under Saline Conditions Experiment" (alpha = 0.10)

Tests of Between-Subjects Effects - Dependent Variable: Cut3 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	70.737	1	70.737	2310.982	.000
	Error	.092	3	.031(a)		
Cultivar	Hypothesis	1.101	11	.100	1.829	.089
	Error	1.805	33	.055(b)		
Block	Hypothesis	.092	3	.031	.560	.645
	Error	1.805	33	.055(b)		
Cultivar * Block	Hypothesis	1.805	33	.055	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =21%
LSD (0.05) – Not Significant
LSD (0.1) = 0.163

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut3	48	.80	1.76	1.2140	.25254	.064
Valid N (listwise)	48					

A28: Analysis of variance for the July 15, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Cut4 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	55.995	1	55.995	713.502	.000
	Error	.235	3	.078(a)		
Cultivar	Hypothesis	.904	11	.082	1.725	.111
	Error	1.571	33	.048(b)		
Block	Hypothesis	.235	3	.078	1.648	.197
	Error	1.571	33	.048(b)		
Cultivar * Block	Hypothesis	1.571	33	.048	.	.
	Error	.000	0	.(c)		

a MS(Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =22%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut4	48	.45	1.54	1.0801	.24013	.058
Valid N (listwise)	48					

Table A29: Analysis of variance for the August 11, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" without the plots exhibiting root rot injury included in data analyzed

Tests of Between-Subjects Effects - Dependent Variable: Cut5 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	34.964	1	34.964	2960.942	.000
	Error	.035	2.972	.012(a)		
Cultivar	Hypothesis	.366	11	.033	2.318	.041
	Error	.345	24	.014(b)		
Block	Hypothesis	.035	3	.012	.823	.494
	Error	.345	24	.014(b)		
Cultivar * Block	Hypothesis	.345	24	.014	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =14%
LSD (0.05) = 0.098

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut5	39	.65	1.26	.9924	.13971	.020
Valid N (listwise)	39					

Table A30: Analysis of variance for the September 8, 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" without the plots exhibiting root rot injury included in data analyzed

Tests of Between-Subjects Effects - Dependent Variable: Cut6 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	34.529	1	34.529	823.278	.000
	Error	.125	2.980	.042(a)		
Cultivar	Hypothesis	.336	11	.031	.833	.611
	Error	.881	24	.037(b)		
Block	Hypothesis	.126	3	.042	1.142	.352
	Error	.881	24	.037(b)		
Cultivar * Block	Hypothesis	.881	24	.037	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =19%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Cut6	39	.75	1.87	.9652	.18789	.035
Valid N (listwise)	39					

Table A31: Analysis of variance for the 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" (alpha 0.05)

Tests of Between-Subjects Effects - Dependent Variable: Total 2008 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	1637.247	1	1637.247	2946.598	.000
	Error	1.652	2.973	.556(a)		
Cultivar	Hypothesis	15.054	11	1.369	2.068	.066
	Error	15.883	24	.662(b)		
Block	Hypothesis	1.668	3	.556	.840	.485
	Error	15.883	24	.662(b)		
Cultivar * Block	Hypothesis	15.883	24	.662	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)

Table A32: Analysis of variance for the 2008 field harvest in the "Forage Production Under Saline Field Conditions Experiment" (alpha 0.10)

Tests of Between-Subjects Effects - Dependent Variable: Total 2008 Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	1637.247	1	1637.247	2946.598	.000
	Error	1.652	2.973	.556(a)		
Cultivar	Hypothesis	15.054	11	1.369	2.068	.066
	Error	15.883	24	.662(b)		
Block	Hypothesis	1.668	3	.556	.840	.485
	Error	15.883	24	.662(b)		
Cultivar * Block	Hypothesis	15.883	24	.662	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =14%
LSD (0.05) – Not Significant
LSD (0.1) = 0.565

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Total 2008 Yield	39	4.98	8.47	6.7571	.92678	.859
Valid N (listwise)	39					

Table A33: Analysis of variance for the two year total dry forage yield per cultivar in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Two Year Total Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	5738.785	1	5738.785	573.848	.000
	Error	29.924	2.992	10.001(a)		
Cultivar	Hypothesis	47.619	11	4.329	1.284	.291
	Error	80.887	24	3.370(b)		
Block	Hypothesis	29.926	3	9.975	2.960	.053
	Error	80.887	24	3.370(b)		
Cultivar * Block	Hypothesis	80.887	24	3.370	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =16%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Two Year Total	39	9.41	17.87	12.7936	2.04510	4.182
Valid N (listwise)	39					

Table A34: Analysis of variance for the two year average total dry forage yield per cultivar in the "Forage Production Under Saline Field Conditions Experiment"

Tests of Between-Subjects Effects - Dependent Variable: Two Year Average Yield

Source		Type III Sum of Squares	df	Mean Square	F	P-value
Intercept	Hypothesis	1434.696	1	1434.696	573.848	.000
	Error	7.481	2.992	2.500(a)		
Cultivar	Hypothesis	11.905	11	1.082	1.284	.291
	Error	20.222	24	.843(b)		
Block	Hypothesis	7.481	3	2.494	2.960	.053
	Error	20.222	24	.843(b)		
Cultivar * Block	Hypothesis	20.222	24	.843	.	.
	Error	.000	0	.(c)		

a 1.004 MS(Block) - .004 MS(Cultivar * Block)
b MS(Cultivar * Block)
c MS(Error)
Coefficient of Variation (%CV) =16%
LSD (0.05) – Not Significant

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Variance
Two Year Avg.	39	4.70	8.93	6.3968	1.02255	1.046
Valid N (listwise)	39					

Table A35: Analysis of variance for Crude Protein (CP) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0027	1	0.0027	0.001006985	0.975309	4.964603
Within Groups	26.8127	10	2.68127			
Total	26.8154	11				

Table A36: Analysis of variance for Acid Detergent Fiber (ADF) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00096783	1	0.000968	0.000439468	0.983687	4.964603
Within Groups	22.02273424	10	2.202273			
Total	22.02370207	11				

Table A37: Analysis of variance for NDF (NDF) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0108	1	0.0108	0.002301563	0.962681	4.964603
Within Groups	46.92463542	10	4.692464			
Total	46.93543542	11				

Table A38: Analysis of variance for RDP (RDP) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.205408333	1	0.205408	0.140828614	0.715296	4.964603
Within Groups	14.58569583	10	1.45857			
Total	14.79110417	11				

Table A39: Analysis of variance for Total Digestible Nutrient (TDN) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.009775521	1	0.009776	0.001928164	0.96584	4.964603
Within Groups	50.69860521	10	5.069861			
Total	50.70838073	11				

Table A40: Analysis of variance for Relative Feed Quality (RFQ) values between FD8 and FD10 cultivars over the length of the 2007 growing season

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.544852083	1	4.544852	0.010104901	0.921916	4.964603
Within Groups	4497.670908	10	449.7671			
Total	4502.21576	11				

Table A41: Analysis of variance for Total Digestible Nutrient (TDN) values between FD8 and FD10 cultivars for Cut 5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21.48401	1	21.48401	9.063534	0.023683	5.987378
Within Groups	14.22228	6	2.370379			
Total	35.70629	7				

Table A42: Analysis of variance for Relative Feed Quality (RFQ) values between FD8 and FD10 cultivars for Cut 5

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1305.094	1	1305.094	4.99811	0.066746	5.987378
Within Groups	1566.705	6	261.1175			
Total	2871.799	7				

Table A43: Regression analysis of GERM experiment versus PROD experiment at the 0.50% saline solution

Regression Statistics	
Multiple R	0.091662796
R Square	0.008402068
Adjusted R Square	-0.027012144
Standard Error	6.893558617
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	11.27445519	11.27445519	0.23725131	0.629990005
Residual	28	1330.592211	47.52115041		
Total	29	1341.866667			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	86.76691183	16.81309703	5.160673948	1.78753E-05
X Variable 1	9.69039811	19.89469612	0.4870845	0.629990005

Table A44: Regression analysis of GERM experiment versus PROD experiment at the 1.00% saline solution

Regression Statistics	
Multiple R	0.061626679
R Square	0.003797848
Adjusted R Square	-0.031780801
Standard Error	4.845323937
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	2.506073027	2.506073027	0.106745134	0.746310436
Residual	28	657.3605936	23.47716406		
Total	29	659.8666667			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	90.21648811	11.81753954	7.634117731	2.57308E-08
X Variable 1	4.568679558	13.98352472	0.326718739	0.746310436

Table A45: Regression analysis of GERM experiment versus PROD experiment at the 1.50% saline solution

Regression Statistics	
Multiple R	0.034474283
R Square	0.001188476
Adjusted R Square	-0.034483364
Standard Error	11.14364437
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.137323358	4.137323358	0.03331693	0.856482559
Residual	28	3477.062677	124.1808099		
Total	29	3481.2			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	77.65297914	27.17887589	2.857107831	0.007973958
X Variable 1	5.870209078	32.16037329	0.182529258	0.856482559

Table A46: Regression analysis of GERM experiment versus PROD experiment at the 2.00% saline solution

Regression Statistics	
Multiple R	0.122552845
R Square	0.0150192
Adjusted R Square	-0.020158686
Standard Error	24.73335283
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	261.1818822	261.1818822	0.426950043	0.518819524
Residual	28	17128.68478	611.7387423		
Total	29	17389.86667			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	71.57236648	60.32359833	1.186473759	0.245405318
X Variable 1	-46.64073232	71.38004708	-0.653414143	0.518819524

Table A47: Regression analysis of FIELD experiment versus GERM experiment

Regression Statistics	
Multiple R	0.497607038
R Square	0.247612764
Adjusted R Square	0.17237404
Standard Error	18.21889593
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1092.384977	1092.384977	3.291028	0.099729814
Residual	10	3319.28169	331.928169		

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	-72.52997158	58.50023383	-1.239823618	0.243346
X Variable 1	10.99956343	6.0633082	1.814119135	0.09973

Table A48: Regression analysis of FIELD experiment versus PROD experiment

Regression Statistics	
Multiple R	0.073469
R Square	0.005398
Adjusted R Square	-0.09406
Standard Error	0.072897
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.000288	0.000288	0.05427	0.820491373
Residual	10	0.05314	0.005314		
Total	11	0.053428			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	0.888558	0.23407	3.796122	0.003508
X Variable 1	-0.00565	0.02426	-0.23296	0.820491

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