DISSERTATION

ENVIRONMENTAL STRESS ASPECTS OF SALTGRASS

[*DISTICHLIS SPICATA* (L.) GREENE]

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2002

Colorado State University

February 28, 2002

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY MOHAMED AHMED SHAHBA ENTITLED ENVIRONMENTAL STRESS ASPECTS OF SALTGRASS [*DISTICHLIS SPICATA* (L.) GREENE] BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

<u>Committee on Graduate Work</u>

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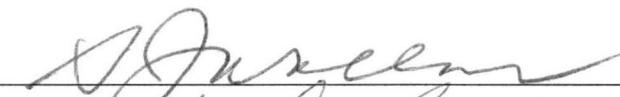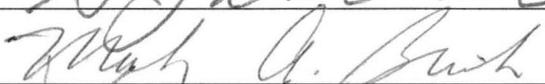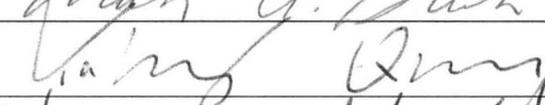
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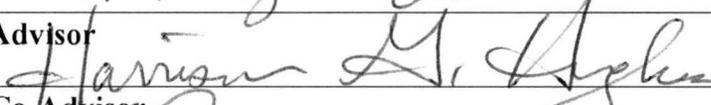
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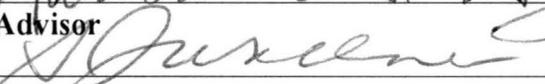
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ABSTRACT OF DISSERTATION

ENVIRONMENTAL STRESS ASPECTS OF SALTGRASS
[*DISTICHLIS SPICATA* (L.) GREENE]

Saltgrass [*Distichlis spicata* (L.) Greene] is undergoing preliminary evaluation at

Colorado State University for use as a turfgrass in adverse environments.

Furthermore, it has a potential as a range species in saline-alkali basins and many of

the salt marsh areas in addition to its importance for wildlife. In cooperation with a

saltgrass breeding project, the objectives of this dissertation work were to: (a)

determine freezing tolerance of saltgrass; (b) determine the relationship between

freezing tolerance and nonstructural carbohydrate content; and (c) determine nitrogen

requirements and to evaluate the nutritive value of saltgrass as affected by N levels.

Stolons of saltgrass accessions were sampled at monthly intervals from October 1999

to April 2000 and from October 2000 through April 2001 and subjected to laboratory

freezing tests. Parts of the sampled stolons were used to assess soluble carbohydrates,

including sucrose, fructose, glucose, raffinose and stachyose using gas

chromatography (GC). Results indicated significant differences among accessions in

LT_{50} (subfreezing temperature resulting in 50% mortality) and carbohydrate content.

Ranking of accessions for LT_{50} (^{o}C) during January, 2000 was A29 = 48 (-20.0) > 55

(-17.0) $\geq$ 32 (-15.5) $\geq$ A65 = C66 (-14.0). In January, 2001 they were ranked with 48

= 55 (-26.0) > A65 = 32 (-23.0) > A29 (-20.0) = C66 (-18.5). Sucrose was the

predominant sugar, but did not show a clear seasonal trend and had no correlation

with freezing tolerance. Fructose, glucose, raffinose and stachyose exhibited clear

seasonal changes, showing highest concentrations during mid-winter. Higher fructose, glucose, or raffinose concentrations were frequently observed in accessions 48, 55, and A29, which coincided with their lowest LT_{50}. In contrast, C66 had the lowest sugar concentrations overall, which related to its sensitivity to lower temperatures.

Accessions A24 and A138 were planted in the field at the Horticulture Research Center, Fort Collins, CO. to determine the nitrogen requirements for these accessions during establishment and to evalute their nutrient content as related to nitrogen level. Results indicated positive linear relationships between cover %, productivity, tissue nitrogen and protein contents with applied nitrogen levels in both seasons. Ca, P and Fe had a positive association while Na, S and Mg had a negative association with nitrogen levels. Establishment in terms of cover and productivity, and nutritive value of the two tested saltgrass accessions increased with increasing N fertilization rate. However, nitrogen had no effect during the first month of establishment on cover when water was critical. Plots which received total N of 450 kg/ha showed the best cover percentage.

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ACKNOWLEDGMENTS

DEDICATION

To the souls of my father and my mother who passed away in 1997 and in 2001

while I was far away working on my degree.

To all Egyptian people who suffered to cover my expenses to finish this work.

<div align="right">

Mohamed Ahmed Shahba
February, 2002
Fort Collins, Colorado, USA

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DISTICHLIS SPICATA (L.) GREENE (SALTGRASS): OVERVIEW

Distichlis spicata (L.) Greene (saltgrass) is a widely distributed, warm season, C_4, dioecious, rhizomatous, perennial grass common in the Atlantic coast tidal areas as well as inland saline marshes of many regions of North America including Mexico. Its rhizomes grow to relatively great depths and are very sharp and armored with numerous silica cells which can penetrate hard clay, shale, or pavement (Hansen et al., 1976). It is quite invasive and can form extensive populations. Saltgrass is being preliminary evaluated for use as turfgrass in some stressed environments. Results thus for have indicated that saltgrass can form dense, low growing sods with great ability to tolerate drought conditions (Fraser and Anderson, 1980; Kopec and Marcum, 2001).

Alpert (1990) has indicated that water sharing occurs among ramets in *D. spicata*. Although this could be of significant benefits, plants have a high investment of mass and nitrogen in underground organs, which might be a cost of clonal growth in desert environments. Saltgrass meadows are found in many climatic zones within the western United States and western Canada. Precipitation ranges from 15 cm in Escalante, Utah, to 66 cm in Oklahoma. Temperature ranges from $-10°$ C to $28°$ C. The number of frost free days varied from 90 days in the northern areas to 207 days in the southern areas of the United States. Because of the vigorous rhizome growth, and asexual reproduction, saltgrass is one of the most versatile plants in the saltgrass meadow. It can directly invade

an existing stand of vegetation by sending out rhizomes. It is also a potential species for revegetation of mine spoils and roadsides in the semiarid west (Pavlicek et al., 1977).

It is usually difficult to correlate vegetation characteristics with environmental factors because of differences in climatic conditions and soil characteristics. Saltgrass meadows are usually lowland areas that are flat to gentle sloping. These areas usually have high water tables at least part of the year and may be subjected to occasional or repeated floodings. Water tables may be found from the ground surface to depths of 3.6 m. The presence of saltgrass usually indicates a clayey soil, good moisture, and high levels of salts. Cluff et al. (1983) suggested that saltgrass seed germination is an episodic event in nature, occurring only when moisture events coincide with optimum seedbed temperatures and can leach sufficient salts to raise moisture potentials above −15 bars.

D. spicata (L.) is a small plant mostly less than 30 cm in height. However, a yield of dry matter of 6,600 kg/ha has been measured for *D. spicata* (L.). Saline saltgrass meadows will become increasingly important for improved pastures because of the need to obtain more forage usable for livestock production. Halophytic grasses have previously been used as food and forage crops. In the dry lake of Texco, near Mexico City, native *D. spicata* (L.) grows in saline soils and serves as the total dietary forage for cattle (Gallagher, 1975). It is an important forage species of the saline-alkali basins of the western United States (Cluff et al., 1983) as well. In many of the salt marsh areas of the interior United States, saltgrass provides the sole forage for cattle during the summer portion of the grazing season. It is considered to be low in nutritive value, however it is palatable to cattle and horses until it becomes mature. Livestock could be maintained on saltgrass if their diet was supplemented with high protein. Also, it is grazed by horses and

cattle along the shores of the Gulf of California (Mexico) near the Colorado river delta. Its seeds contain approximately 14 % protein and are small (average about 0.8 mg each) but are produced in abundance. It thus is an important species for wildlife such as waterfowl and the Florida salt marsh vole (*Microtus pennsylvaicus duckecampbelli*), which is on the Endangered and Threatened Species List of Southern United States. Ducks eat the dried seeds and wild geese eat the tender forages. It is also significant in the salt marshes where it provides nesting grounds for birds, fish and larvae of many species of marine invertebrate animals. As salt marsh plants decompose, their stored nutrients provide a steady source of food for clams, crabs, and fish (USDA, NRCS, National Plant Data Center).

Most of the work on the genus *Distichlis* is restricted to older literature. Nielson (1956) studied the variability of *D. spicata* in western United States to provide information for establishing a selective breeding program on *D. spicata*, so that the plant might be used more effectively in saline or marshy areas. Anderson (1974) reviewed the structure of this plant. Hansen et al. (1976) monitored the environmental parameters - air temperature, relative humidity, and the soil conditions (pH, moisture content, individual ion concentrations and their total osmotic effect) in a typical *D. spicata* community during the plant's normal growing season. They also discussed changes in internal concentrations of ions, crude protein, and growth as affected by environmental conditions both in the field and in the laboratory, and presented the basic structural features of the plant that favor its existence in saline habitats.

D. spicata is selenium tolerant (Wu et al., 1997) and could be used for reclamation of disturbed and contaminated sites (Dahlgren et al., 1997). It is one of the most salt

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tolerant graminoides frequently associated with salt marshes and inland saline wetlands, and its response to soil salinity has been the subject of a number of growth-chamber and greenhouse investigations. It shows a very broad distribution with respect to soil salinity. However, it is most commonly found in areas of moderate soil salinity (250 – 425 mM) (Taylor, 1939; Hansen et al., 1976). It can grow vigorously on wet, saline soils where most others will not survive (Nielson, 1956). Laboratory growth studies, of *D. spicata* from inland marshes have indicated the greatest growth at soil salinities of about 250 mM (Hansen et al., 1976). However, Adams (1963) observed that optimal NaCl levels for greenhouse-grown *D. spicata* from North Carolina marshes was about 170 mM. Kemp and Cunningham (1981) reported that growth rates of *D. spicata* under high light intensity in controlled environment chambers were reduced significantly by hydroponics solutions containing up to 500 mM NaCl. Hansen et al. (1976) presented field observations that indicated that *D. spicata* is one of the most tolerant grasses in the Atlantic tidal marsh community and may well require some salt for optimal growth. Several reports have indicate little growth inhibition at salinities of 25-30 parts per thousand (ppt) (Adams, 1963; Kemp and Cunningham 1981; Parrondo et al., 1978; Taylor, 1938). Others have found significant growth reduction at 20 ppt (Tiku, 1976), and higher productivity with fresh water vs. marine water (Smart and Barko, 1980). Optimum soluble salts for *D. spicata* was determined to be 15,000 ppm by Hansen et al. (1976). Warren and Brockelman (1989) found a negative correlation between photosynthesis and soil salinity in field and greenhouse plants. In contrast, leaf transpiration had no correlation with soil salinity in either group of plants. Sodium ion secretion rates by leaf salt glands is found to have a significant correlation with soil salinity. They attributed

reduced photosynthesis, and increased leaf respiration to the reduced stem growth found with increasing soil salinity.

Smart and Barko (1980) found a salinity effect on the growth of *D. spicata*. They suggested a selective uptake of potassium and exclusion of sodium. Numerous studies have dealt with the effects of salinity on physiology, morphology, and growth of plants (Bernstein and Hayward, 1958; Flowers, et al., 1977; Kemp and Cunningham, 1981; Longstreth and Noble, 1979; Poljakoff-Mayber and Gale, 1975). Their conclusions were different according to interspecific differences in response to salinity and to differential responses resulting from interaction of salinity with other environmental factors. For example, humidity (Gale et al., 1970), irradiance (Longstreth and Strain, 1977; Mallot et al., 1975), nitrogen levels (Haines and Dunn, 1976), and light and temperature (Kemp and Cunningham, 1981) can all affect plant response to salinity. The most widely reported responses to salinity is an increase in leaf thickness (Jennings, 1976; Longstreth and Noble, 1979; Poljakoff-Mayber, 1975; Waisel, 1972). Increased succulence appears to have beneficial effects on CO_2 exchange by increasing the internal surface area per unit leaf surface over which CO_2 diffusion can occur. The effect of light, temperature, and salinity on growth, net CO_2 exchange, and leaf anatomy of *D. spicata* were investigated by Kemp and Cunningham (1981) in controlled environment chambers. Their results indicated that growth rates were significantly reduced at low light, high substrate salinity, or low temperature; however, temperature and salinity had no effect on growth rates at high light. This indicated that, the mechanisms by which *D. spicata* tolerates salt appear to be closely coupled to the utilization of light energy.

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Salts of sodium chloride and sodium carbonate have greater adverse effects on saltgrass than salts of sodium sulfate (Ahi and Powers, 1938). Osmotic pressure varies directly with the salinity concentration of soil water. Saltgrass adjusts to higher salt concentrations in the soil by adjusting internal osmotic potential (Daubenmire, 1948; Salisbury and Ross, 1969). Ahi and Powers (1938) noted that the total weight of dry matter of *D. spicata* plants obtained in the highest concentration of sea water at 13°C was more than three times greater than plants grown at 21°C at similar salt concentrations. Chapman (1974) suggested that in cold weather *D. spicata* grows better on non-saline soils, whereas in hot weather it does better on saline soils. Hansen et al. (1976) found that the maximum growth for *D. spicata* was obtained at 15,000 ppm soluble salt in nutrient solution cultures in laboratory experiments. Nearly equal concentrations of sodium and potassium were found in the plant tissue where the growth of the plants was optimal. Such a ratio was maintained in the plants during most of the growing season. In the field, the greatest amount of growth of saltgrass takes place when temperatures are cool and soil moisture is quite high during the early spring. During periods of high salt and water stress, morphological and anatomical adaptations of the stomata, saltglands, and trichomes of saltgrass are important for survival. In a recent study, Alshammary (2001) tested growth responses of saltgrass to salinity in a container and in hydroponic experiments. He found a 50 % saltgrass shoot growth reduction at 34.5 dS/m and a 50 % root growth reduction at 40.8 dS/m. There was no injuries in saltgrass although leaf firing and root cortex cell collapse were noticed in Kentucky bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea* Schreb) and alkaligrass [*Puccinellia distans* (L.) Parl.].

The effects of freezing temperatures on living organisms have long interested biologists, both from practical and theoretical points of view (Levitt, 1980). Freezing tolerance is a significant environmental adaptation that control plant geographical distribution. The freezing resistance of warm-season turfgrass has been researched in bermudagrass (*Cynodon spp.*) (Anderson et al., 1988 and 1993; Ibitayo et al., 1981); zoysiagrass (*Zoysia spp*) (Dipaola and Beard, 1992; Dunn et al., 1999; Rogers et al.; 1975 and 1977;), centipedegrass [*Eremochloa ophiruoides* (Munro) Hackel] (Fry et al., 1993; Johnsten and Dickens, 1976 and 1977; Palmertree et al., 1973; Walker and Ward, 1974), St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] (Fry et al., 1991; Maier and Lang, 1994), seashore paspalum (*Paspalum vaginatum* Swartz) (Cardona et al., 1997), and buffalograss [*Buchloe dactyloides* (Nutt.) Engelm] (Qian et al., 2001). However, no research results related to saltgrass freezing tolerance have been reported.

Nonstructural carbohydrates (NSC) are thought to serve an important role in freezing tolerance of many plants including at least some warm-season turfgrasses (Levitt, 1980; Fry et al., 1993). In contrast some studies indicated no correlations or relationships between this adaptation and NSC composition (Bush et al., 2000; Dunn and Nelson, 1974; Fry et al., 1991; Maier and Slang, 1994). Information is lacking regarding the changes of endogenous soluble carbohydrates of saltgrass [*Distichlis spicata* (L.) Greene] during cold acclimation.

It is well known that the productivity of North American salt marshes is limited by the availability of nitrogen (Gallagher, 1975; Squiers and Good, 1974; Valiela and Teal 1974). Evidence for nitrogen limitation has been based predominantly on the response of the vegetation to nitrogen fertilization (Gallagher, 1975; Patrick and Delaune, 1976;

Sullivan and Daiber, 1974; Valiela and Teal, 1974). Smart and Barko (1980) investigated

D. spicata response to different nitrogen concentrations on different sediments (fresh

water, brackish, and marine sediments. They reported a growth limitation due to nitrogen

on most sediments. It is important, however, to evaluate the nitrogen nutrition of salt

marsh plants and how it affects its establishment and productivity. We know of no studies

dealing with this issue.

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CHAPTER ONE

COLD HARDINESS OF SIX SALTGRASS ACCESSIONS

ABSTRACT

Freezing tolerance is an environmental adaptation that significantly influences plant geographical distribution. It therefore is important to understand the range of freezing tolerance of any potential turfgrass so one may assess its likely use. The objective of this study was to determine the relative freezing tolerance, seasonal changes in the freezing tolerance, and winter survival of six accessions of saltgrass [*Distichlis spicata* (L.) Greene]. Saltgrass accessions A65 and A29 were collected from the Front Range of Colorado while C66 and 32 from Nevada, and 55 and 48 from South Dakota. These accessions were established in a field nursery in Fort Collins, CO. Stolons of these accessions were sampled at monthly intervals from October 1999 through April 2000 and from October 2000 through April 2001 and subjected to laboratory freezing tests. No differences among accessions in LT_{50} (subfreezing temperature resulting in 50% mortality) were found in October in both seasons. Cold hardiness of the saltgrass accessions increased gradually during the fall with maximum hardiness at midwinter. During midwinter, freezing tolerance was significantly different among accessions. Ranking of accessions for LT_{50} (°C) during January, 2000 was A29 = 48 (-20.0) > 55 (-

17.0) $\geq$ 32 (-15.5) $\geq$ A65 = C66 (-14.0). In January, 2001 they were ranked with 48 = 55 (-26.0) > A65 = 32 (-23.0) > A29 (-20.0) = C66 (-18.5). In December and January sampling dates of the first season, accessions A29, 48 and 55 exhibited the highest relative regrowth when exposed to temperatures $\approx$ -20.0°C. In midwinter of the second season, accession 55 showed the highest regrowth after being subjected to temperatures $\approx$ -25.0°C. C66 had the lowest regrowth potential after freezing treatments in both seasons. Winter survival in the field correlated negatively with LT_{50} value with accessions 48, A29, and 55 demonstrating greater winter survival while C66 was the least. The difference in freezing tolerance among accessions may be in part associated with their origin-inherited adaptation. This information would be useful in saltgrass breeding projects to select and develop freezing tolerant saltgrass.

INTRODUCTION

The effects of freezing temperatures on living organisms have long interested biologists, both from practical and theoretical points of view (Levitt, 1980). Freezing tolerance is a significant environmental adaptation that controls plant geographical distribution. Freezing stress may be defined as the freezing potential of the low temperature stress. The simplest measure of freezing stress is the number of degrees the environmental temperature is below the freezing point of pure water at atmospheric pressure. The freezing point of a plant is always below that of pure water. Extracellular freezing leads to secondary water stress, resulting in a dehydration strain, due to the efflux of the cell's water to the ice nuclei in the intercellular spaces. The cell then desiccates in exactly the same manner as if the water were removed by evaporation. As the temperature drops and the freeze-dehydration increase, the cell contents became more and more concentrated. In nature, cooling occurs slowly, and the freeze-dehydration steadily lowers the freezing point of the remaining cell sap, maintaining it at approximately the prevailing temperature, in this way avoiding intracellular freezing. Direct injury due to the freezing process, can occur only as a result of intracellular freezing.

The freezing resistance of warm-season turfgrass has been researched in bermudagrass (*Cynodon spp.*) (Anderson et al., 1988 and 1993; Ibitayo et al., 1981); zoysiagrass (*Zoysia spp*) (Dipaola and Beard, 1992; Dunn et al., 1999; Rogers et al.; 1975 and 1977), centipedegrass [*Eremochloa ophiruoides* (Munro) Hackel] (Fry et al., 1993; Johnston and Dickens, 1976 and 1977; Palmertree et al., 1973; Walker and Ward, 1974), St. Augustinegrass [*Stenotaphrum secundatum* (Walt) Kuntze] (Fry et al., 1991; Maier et

al., 1994), seashore paspalum (*Paspalum vaginatum* Swartz) (Cardona et al., 1997), and buffalograss [*Buchloe dactyloides* (Nutt.) Engelm] (Qian et al., 2001). However, saltgrass has not been subjected to freezing tolerance tests.

Zoysiagrass was found to have freezing tolerance of LT_{50} ranging from -8 to $-14°C$. Electrolyte leakage and regrowth studies by Anderson et al. (1988 and 1993) with bermudagrass indicated that the LT_{50} ranged from -7°C for 'Tifgreen' to -11°C for 'Midiron'. Furthermore, Ibitayo et al. (1981) study of 'Brookings' bermudagrass indicated survival at -17°C at Fort Collins, Colorado. Qian et al. (2001) found that the LT_{50} of six buffalograss cultivars ranged from $-21.7°C$ for Tatanka and 91-118 to $-9.2°$ C for UCR-95 during midwinters.

Distichlis spicata (L.) Green (saltgrass) is a widely distributed, rhizomatous, perennial grass common in the Atlantic coast tidal areas as well as inland saline marshes of many regions of North America including Mexico (Hansen et al., 1976). Because of the vigorous rhizome growth, and asexual reproduction, saltgrass is one of the most versatile plants in the saltgrass meadow. It can directly invade an existing stand of vegetation by sending out rhizomes. It is a potential species for revegetation of mine spoils and roadsides in the semiarid west (Pavlicek et al., 1977).

Saltgrass meadows are found in many climatic zones within the western United States and western Canada. Precipitation in these areas range from 15 cm in Escalante, Utah, to 66 cm in Oklahoma. The number of frost-free days vary from 90 days in the northern areas to 207 days in the southern areas of the United States. The presence of saltgrass usually indicates a clayey soil, good moisture, and high levels of salts. *Distichlis spicata* is one of the most salt tolerant graminoides and is frequently associated with salt

marshes and inland saline wetlands. Its response to soil salinity has been the subject of a number of growth-chamber and greenhouse investigations (Adams, 1963; Kemp and Cunningham 1981; Hansen et al., 1976; Parrondo et al., 1978; Smart and Barko, 1980; Taylor, 1938; Tiku, 1976; Warren and Brockelman, 1989).

Saltgrass is an important forage species of the saline-alkali basins of the western United States (Cluff et al., 1983). In many of the salt marsh areas of the interior United States, saltgrass provides the sole forage for cattle during the summer portion of the grazing season. It is low in nutritive value but, is palatable to cattle and horses until it becomes mature. Livestock could be maintained on saltgrass if their diet is supplemented with high protein.

Currently, a saltgrass breeding project has been initiated in the Dept. of Horticulture and Landscape Architecture for the purpose of the development of turftype lines. Saltgrass is being preliminary evaluated for use as turfgrass in some stressed environments. Results indicated that saltgrass can form dense, low growing sods with great ability to tolerate drought conditions (Fraser and Anderson, 1980; Kopec and Marcum, 2001). No research results related to saltgrass freezing tolerance are available. LT_{50} data and winter survival observations would be valuable for accurately assessing the freezing tolerance of saltgrass accessions. This would be valuable for breeders for the purpose of the selection of lines for regional use.

The objective of this study was to determine the relative freezing tolerance, seasonal changes in freezing tolerance, and winter survival of six accessions of saltgrass.

MATERIALS AND METHODS

Saltgrass accessions used in this experiment were A65, A29 (collected from the Front Range of Colorado), C66, 32 (collected from Nevada), 55 and 48 (collected from South Dakota). These accessions have been established in field plots at the Horticulture Research Center, Fort Collins, CO. Irrigation was provided once a year in early August by flooding the field. No fertilizers were applied before or during sampling dates. Grasses were unmowed. Daily maximum and minimum air temperature were recorded via the cellular-linked weather station located 100 m east of the study area.

Laboratory test

Accessions used in this experiment were sampled at monthly intervals from October 1999 through April 2000 and from October 2000 through April 2001. On each sampling date, stolons were collected from each of the two replicated plots for each accession (four replications were sampled in the second season). After washing them with cold water to remove soil and plant debris, stolons were divided into 7-8 fractions. Each fraction, which contains at least 10 nodes, was individually wrapped in moist tissues and then placed in aluminum foil for targeted freezing temperatures. Samples were subjected to low temperature treatments using a thermo-controlled freezer (Tenny Jr. Programmable Freezer, Tenny Inc., South Brunswick, NJ). The freezing chamber was programmed to cool linearly at 2°C/h after an initial 16 hour at 0°C. One fraction of stolons was removed at each target temperature. Target temperatures (3°C intervals) were varied with sampling dates, air temperatures and expected acclimation to cover the range of expected 50% lethal temperatures. Thermocouples were inserted inside the wrapped samples to measure the actual temperature of the samples and compare it with the chamber temperatures. Samples were thawed overnight at 2°C as soon as they were removed from the freezing chamber. Nonfrozen controls were kept at 2°C during the freezing treatment. Following

thawing, individual nodes were planted in a foam cone (3 cm inside diameter by 8 cm deep) filled with commercial potting soil. All plants were maintained in the greenhouse at approximately 25°C. Irrigation was applied by a mist system to provide about 3-5 mm/day.

Saltgrass response to freezing temperature was evaluated based on stolon survival and total stolon regrowth. Survival was recorded by observing regeneration of shoots 4 wk after planting. Total stolon regrowth was harvested 8 wk after planting, and dried at 70°C for 3 days to determine dry mass. The relative total stolon regrowth was calculated as a % of each accession's 2°C control. Using relative total regrowth rather than an absolute growth value as a measure of response to freezing temperature avoids the genetic differences in growth rates among different accessions.

Field evaluation:

To evaluate saltgrass response to seasonal climatic changes, leaf color was visually rated using a scale of 1 to 9 (9 = green and 1= brown) over the sampling dates. In April and May 2000 and 2001 winter survival of saltgrass plots in the field were assessed by visually rating the percentage of the plot area that exhibited green-up.

Data analysis:

The PROC PROBIT procedure of the statistical analysis system (SAS Institute, 1989) was used to predict LT_{50}, which can be defined as the sub-freezing temperature that resulted in 50% survival. The lower the LT_{50} the greater the freezing tolerance of the accession. Likewise, regression analysis was used to determine the relationships between total stolon regrowth and freezing temperature treatments within each sampling date. The generated regression equations were used to predict the freezing temperature that caused 50% total stolon regrowth reduction (GT_{50}). The LT_{50} and GT_{50} of each replication were subjected to the analysis of variance (ANOVA) (SAS Institute, 1989) as response

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variables to test the differences among accessions over sampling dates. A significant interactions between accessions and sampling dates were found. Therefore, mean separations among accessions within sampling dates were performed at

$P = 0.05$ by Fisher's LSD test. Winter survival in the field was subjected to the analysis of variance, and means were separated by Fisher's LSD at $P = 0.05$.

RESULTS

Seasonal lethal low temperature

October 1999 to April 2000. In October, all accessions showed good survival from −5 to −8°C while no survival was detected at −11°C (Fig. 1.1). All accessions showed a temperature decrease for LT_{50} from October to January as related to lower air temperatures and acclimation (Fig. 1.3). This decrease in LT_{50} value was negatively correlated with fall color retention (r = -0.74) (Table 1.1). Accessions were significantly different in their LT_{50} during midwinter (Fig. 1.1). Lowest individual LT_{50} for this season was −20°C, shown by accessions A29 and 48 in January 2000. From February to April, all accessions showed an increase in LT_{50} with a significant difference among them during February and March but there was no difference between accessions in April. Ranking of accessions for LT_{50} (°C) during January of the first season was A29 = 48 (-20.0) > 55 (-17.0) ≥ 32 (-15.5) ≥ A65 = C66 (-14.0) (Fig. 1.1).

October 2000 to April 2001. In October, LT_{50} values of all accessions ranged from −11.0 to −14.0°C with no significant difference among them (Fig. 1.2). In November, significant differences among accessions were detected with the lowest LT_{50} for 48 and 55 (-20.0°C), and the highest exhibited by 32 and C66 (-14.0°C). In January, 55 and 48 achieved the lowest LT_{50} of −26.0°C. January LT_{50} ranking was 48 = 55 (-26.0°C) > A65 = 32 (-23.0°C) > A29 (-20.0°C) = C66 (-18.5°C). LT_{50} started to increase in February and March without significant differences among accessions. They ranged from −11.0 to −14.0°C. In April, LT_{50} increased for all accessions except 55, which remained at −14.0°C.

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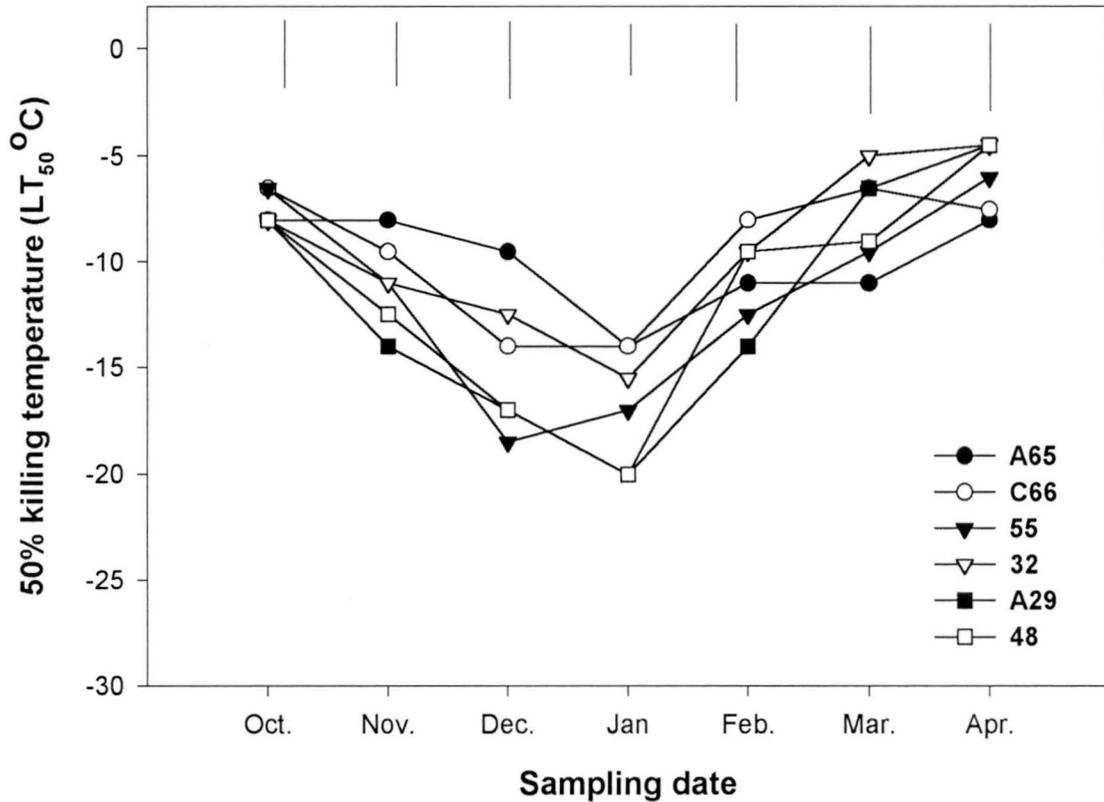
Fig. 1.1. Seasonal patterns of LT_{50} of six saltgrass accessions sampled monthly from Oct. 1999 to April 2000. Vertical bars at the top indicate LSD (P = 0.05) for accession comparison within each date.
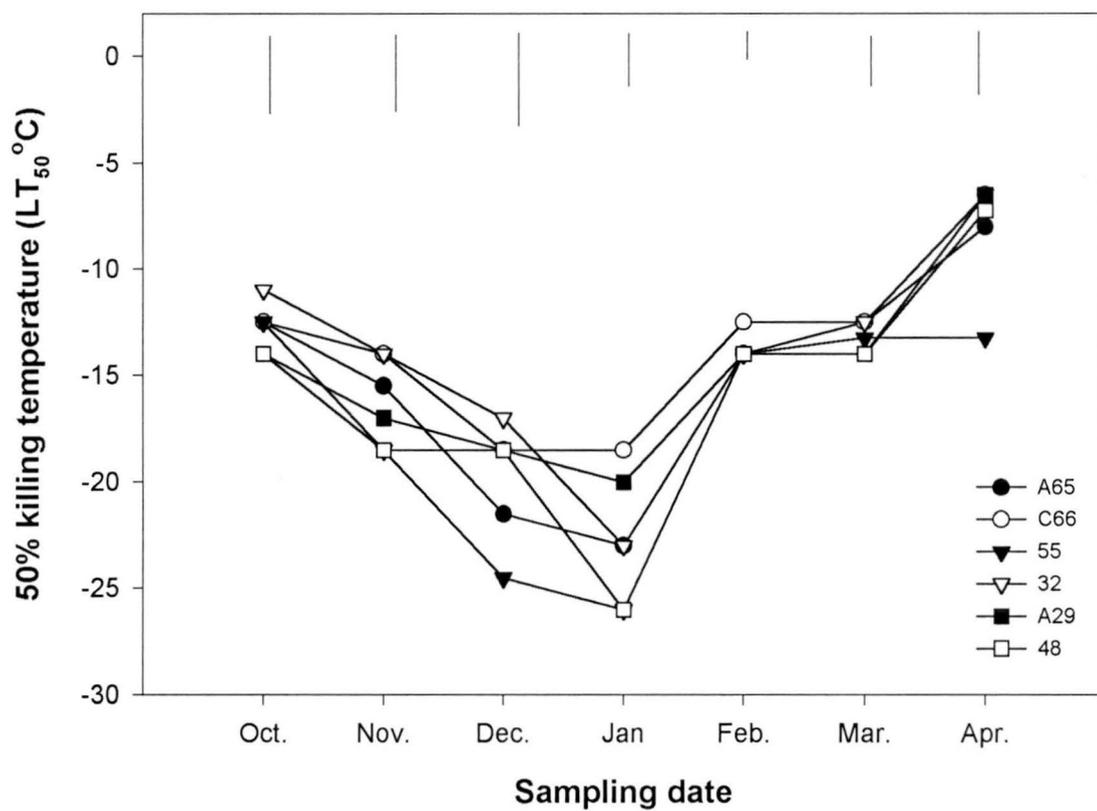
Fig. 1.2. Seasonal patterns of LT$_{50}$ of six saltgrass accessions sampled monthly from Oct. 2000 to April 2001. Vertical bars at the top indicate LSD (P = 0.05) for accession comparison within each date.
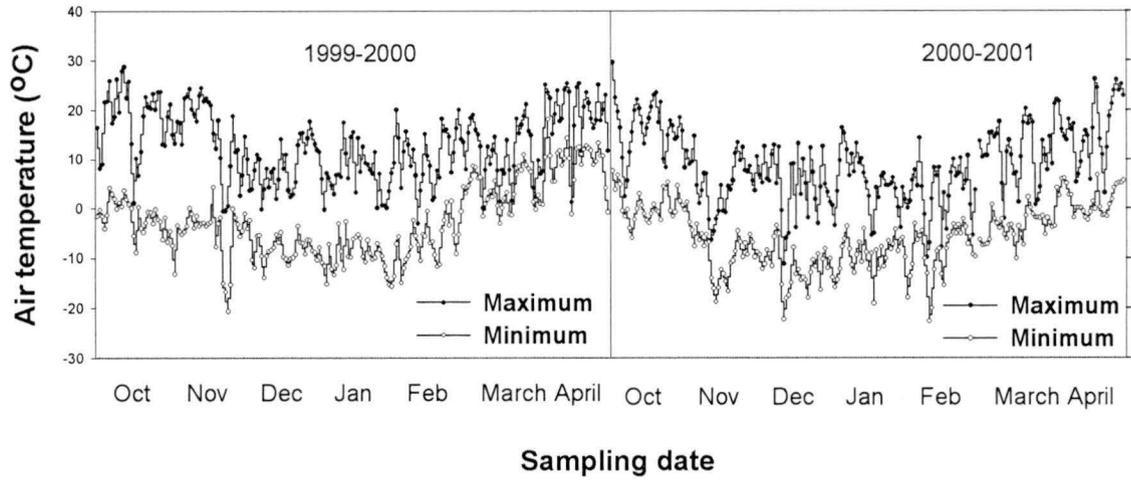
Fig. 1.3. Minimum and maximum air temperatures in Fort Collins, CO. from Oct. to April in 1999-2000 and 2000-2001.

Total stolon regrowth

Regression analysis indicated a significant linear temperature effect on total stolon regrowth for all accessions over all sampling dates in both seasons with R^2 values ranging from 0.70 to 0.98. Over the two seasons, there were significant differences in temperature that caused a 50% total regrowth reduction among saltgrass accessions (Figures 1.4 and 1.5).

1999-2000. In October, the temperature that caused a 50% growth reduction (GT_{50}) was $-10.4°C$ for A29 and 48, which were significantly lower than other accessions. Regression predicted that C66 had a GT_{50} of $-7.2°C$. In November, the GT_{50} of A29 was $-16.5°C$, which was the lowest among all accessions. In December, 55 had a GT_{50} of $-21.0°C$ while that of A29 remained at $-16.1°C$. The lowest GT_{50} overall for this season was $-21.9°C$ for accession A29 in January. Ranking of accessions for GT_{50} was: A29 ($-21.9°C$) < 48 ($-19.2°C$) ≤ 55 ($-17.5°C$) = 32 ($-17.2°C$) < A65 ($-13.7°C$) < C66 ($-11.2°C$) (Fig. 1.4). GT_{50} increased from February to April with a significant difference among accessions. In April, GT_{50} was $-6.3°C$ for A29, which was lower than 32, 48, 55 and C66 ($≈ 6.0°C$) and higher than A65 ($-8.1°C$).

2000-2001. Accessions were more cold hardy and exhibited greater levels of regrowth than the first season (Fig. 1.5). Also, some accessions acclimated differently because of the lower air temperature, which prevailed during 2000-2001 (Fig. 1.3). In October, the GT_{50} of 32 was $-12.0°C$, which was higher than other accessions ($-15.6°C$). In November, the GT_{50} was $≈ -19.0°C$ for 48 and 55, which were the lowest among all accessions. In midwinter 55, 48 and A29 exhibited lower GT_{50} than the other accessions. In April, the lowest GT_{50} was $-10.9°C$ (55), which was lower than all other accessions ($≈ -6.4°C$).
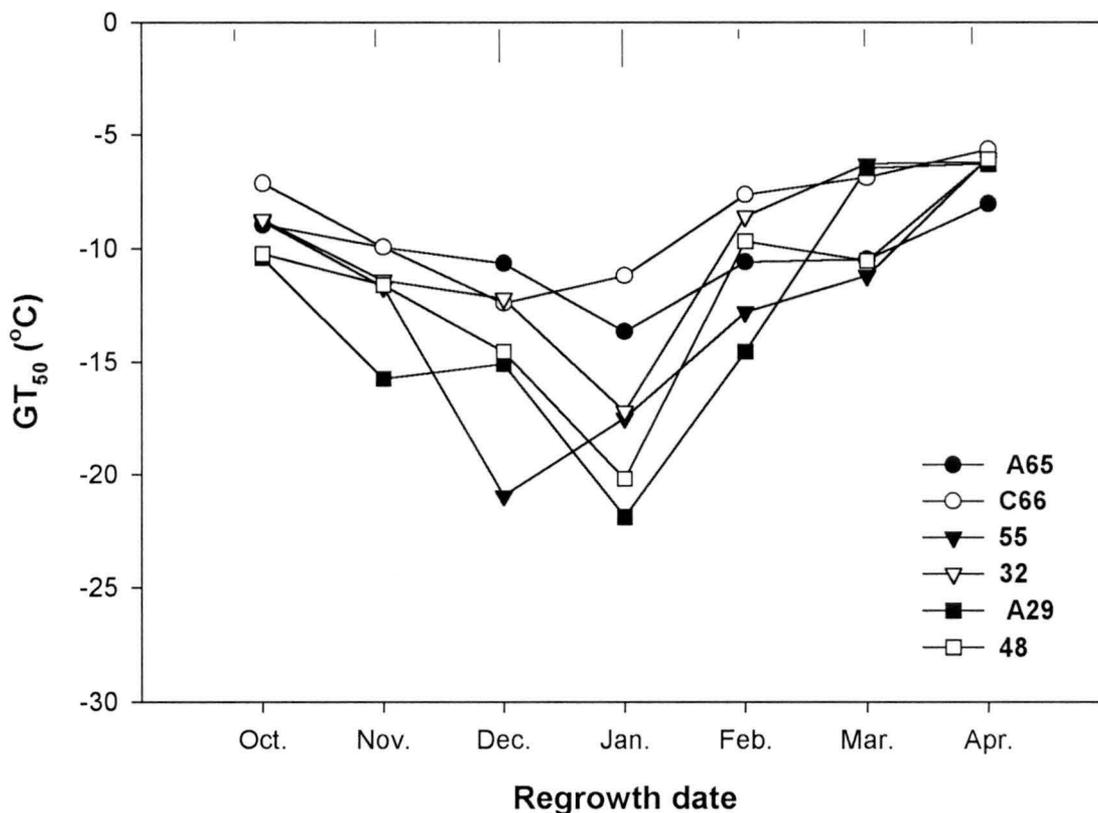
Fig. 1.4. Seasonal patterns of minimal temperatures caused 50% relative total stolons regrowth reduction (GT$_{50}$) of six saltgrass accessions sampled monthly from Oct. 1999 to April 2000. Vertical bars on the top indicate LSD (P = 0.05) for accession comparison within each date.
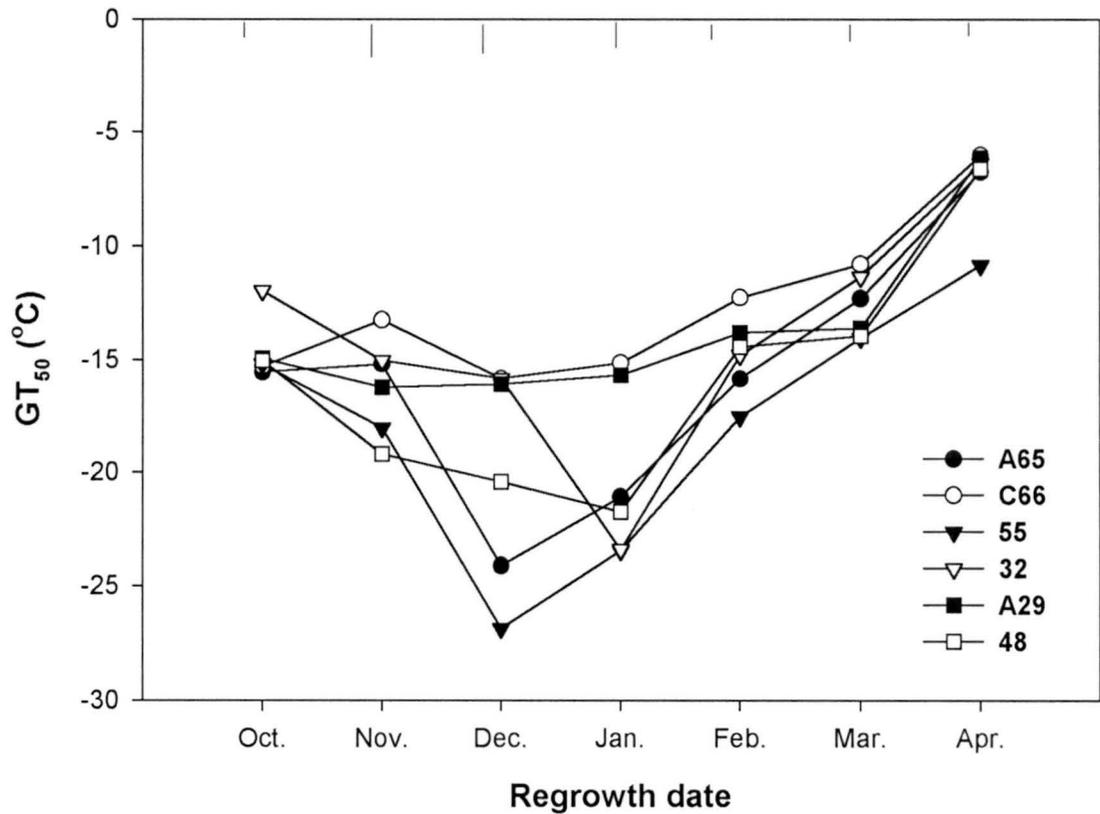
Fig. 1.5. Seasonal patterns of minimal temperatures caused 50% relative total stolons regrowth reduction (GT_{50}) of six saltgrass accessions sampled monthly from Oct. 2000 to April 2001. Vertical bars on the top indicate LSD (P = 0.05) for accession comparison within each date.

Winter survival in the field

There were significant differences in winter survival in the field between seasons and among accessions. C66 showed the lowest winter survival (85% in the first season and 72% in the second). On the other hand, 48 and A29 were the highest in the first season (94 and 92%). Ranking of accessions based on LSD mean separation test was: 48 (94%) = A29 (92%) = 55 (90%) ≥ 32 (87.5%) = A65 (87.5%) = C66 (85%) (Table 1.1).

In the second season, the ranking of accessions for winter survival was 48 (86%) = 55 (85%) = A29 (84%) ≥ 32 (82%) = A65 (80%) > C66 (72.5%) (Table 1.2).

Color retention was negatively correlated with LT_{50} over season for each accession. There were significant differences in fall color retention and spring green up among accessions, but these differences were not clearly associated with the differences in their freezing tolerance. All accessions stopped growth, turned brown in late November and early December (Tables 1.1 and 1.2).

Table 1.1. Winter survival and color of the tested saltgrass accessions grown in the field during the first season of the study (1999-2000).

| Accessions | Winter survival[+] (%) | Color[++] 1-9 | | | | |
		Oct.	Nov.	Dec. to Feb.	March	April
A65	87.5bc	5.2d[*]	4.0c	1	3	4.0b
C66	85c	6.0c	3.0d	1	2	3.0c
55	90ab	7.0b	4.0c	1	3.2	4.0b
32	87.5bc	7.0b	5.0b	1	2.9	4.8a
A29	92a	8.0a	6.1a	1	3.0	5.0a
48	94a	6.0c	4.0c	1	3.0	4.0b

[+] Winter survival was estimated visually in May as a green-up percentage for each sampled plot.

[++] Color was rated visually on a 1 to 9 scale where 1 = brown and 9 = green.

[*] Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ using Fisher's LSD test.

Table 1.2. Winter survival and color of the tested saltgrass accessions grown in the field during the second season of the study (2000-2001).

Accessions	Winter survival[+] (%)	Color[++] 1-9				
		Oct.	Nov.	Dec. to Feb.	March	April
A65	80c	4.0d[*]	2.4c	1	1.4	2.6b
C66	72.5d	5.2c	2.0d	1	1.6	2.0c
55	85a	5.8b	3.2c	1	2.0	2.4b
32	82bc	5.8b	3.8b	1	1.3	3.6a
A29	84ab	7.0a	4.1a	1	2.2	4.2a
48	86a	5.4c	2.8c	1	1.4	2.8b

[+] Winter survival was estimated visually in May as a green-up percentage for each sampled plot.

[++] Color was rated visually on a 1 to 9 scale where 1 = brown and 9 = green.

[*] Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ using Fisher's LSD test.

DISCUSSION

In both seasons, there was no significant difference in freezing tolerance among all accessions in October as tissues were nonacclimated (Fig. 1.1). Studies on different grasses indicated that freezing tolerance is dependent on the rate of acclimation as well as the level and stability of hardiness (Qian et al., 2001). Fry et al. (1993) found that acclimated centipedegrass stolons can tolerate and survive lower freezing temperatures than post-acclimated stolons. Cardona et al. (1997) indicated a difference in freezing tolerance between acclimated and non-acclimated paspulum ecotypes in addition to the presence of variability among acclimation traits within the species.

In our study, we had two seasons with quite different air temperatures. Figure 1.3 showed the change in minimum and maximum daily air temperature from October to April in 1999-2000 and 2000-2001. The second season was relatively cooler than the first season (Fig. 1.6A-B). This clear variability could be related to the variation in cold hardiness of the same accessions from season to season. Figure 1.6C-H illustrates the difference in freezing tolerance between the two seasons for each accession in relation to the change in air temperature.

In both seasons, A65 became dormant earlier (Tables 1.1 and 1.2), acclimated faster at relatively high temperatures giving the highest relative total regrowth at -5 and -8°C. However, its hardiness demonstrated lower stability at lower temperatures (Fig. 1.1 and 1.2). A29, 55, and 48 maintained their vitality longer and had higher freezing tolerance. A29 and 48 were the most cold hardy in the first season, down to -20°C (Fig. 1.1). In the second season, A29 exhibited lower tolerance while 55 and 48 were superior with an LT_{50} approaching -26°C (Fig. 1.2).
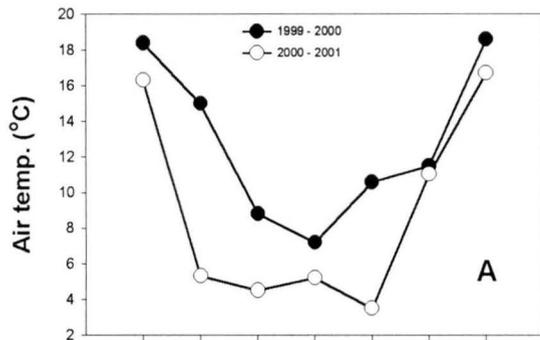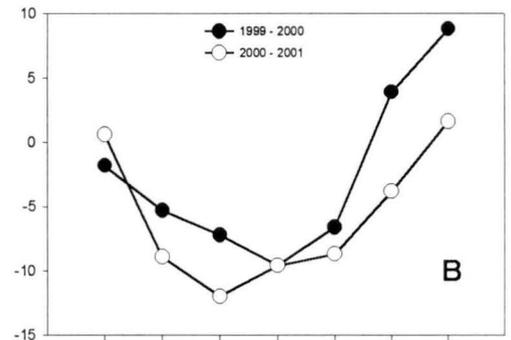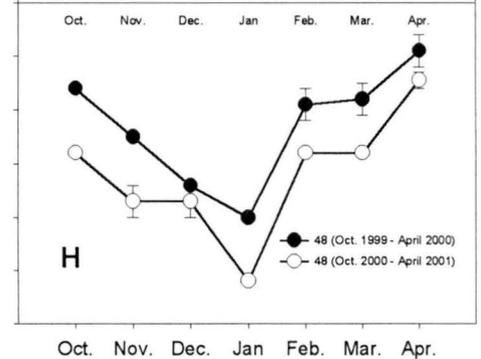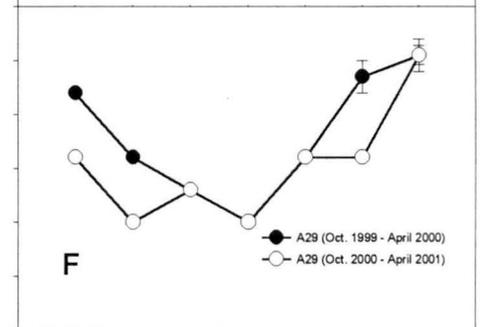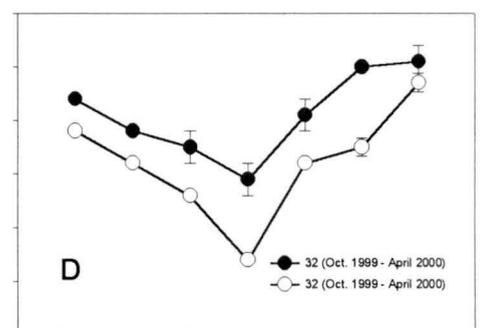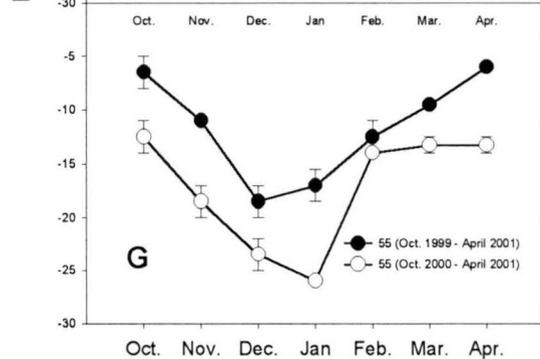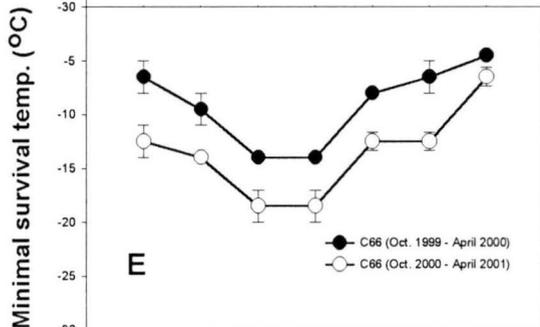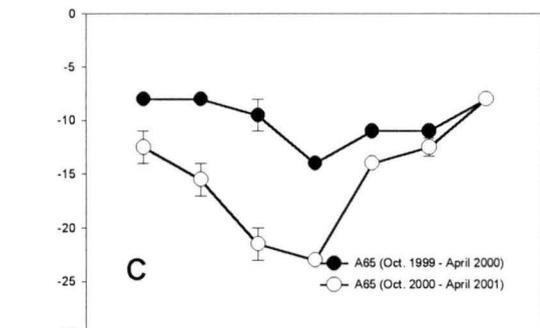
Fig. 1.6. Change of seasonal patterns on minimal survival temperatures of the same saltgrass accession in response to the change in air temperature from Oct. 1999 to April 2001. Bars represent standard errors.

During midwinter, considerable variation in freezing tolerance among accessions was noted with a difference in ranking between the two seasons. This variation could be related to the variation in air temperature that prevailed and the difference in the lowest temperature required to achieve the maximum and more stable acclimation level. Based on this idea we may explain that A29 was the hardiest overall during the first season, to $-20°C$, while 55 and 48 were the hardiest in the second season, to $-26°C$.

Differences in freezing tolerance could be related to the origin of each accession. Ranking of accessions for mean LT_{50} during midwinter of the first season was A29 $\geq$ 55 = 48 > A65 = 32 $\geq$ C66. In midwinter of the second season they were ranked with 55 > 48 = A65 $\geq$ 32 $\geq$ A29 = C66. There was a decrease in LT_{50} values ranging from 6 to 9°C, except for A29. This indicates that A29 achieved its maximum acclimation during the first season, while others required cooler air temperatures to achieve greater acclimation. The sensitivity of C66 over the two seasons could be explained by its origin in Nevada, which is relatively warm, i.e. this accession may be genetically adapted to warmer climates, and thus not be as cold hardy. Although 32 was collected from Nevada also, it seems to be better adapted to cooler climates than C66.

Total stolon regrowth was reduced linearly by decreasing treatment temperature. The lowest temperature resulted in a 50% reduction in total stolon regrowth followed the same trend of LT_{50} over all sampling dates (Figures 1.4 and 1.5). Accordingly, we can conclude that saltgrass accessions survive severe lower temperature although they lose some of their vitality. Also, the negative correlation between cold hardiness and color retention indicates physiological and biochemical changes inside the stolons, which are associated with acclimation and survival.

In summary, our results suggest that 55 and 48 have greater freezing tolerance at lower temperatures while A29 has demonstrated greater freezing tolerance at moderately lower temperatures as compared to the other tested accessions. C66 had poor freezing tolerance. C66 cannot be recommended as a turf in Colorado but the other more cold tolerant accessions are likely to survive the lowest expected temperature. Further study would be recommended to determine the mechanisms of saltgrass freezing tolerance, which could include conversion of membrane stability (Cyril et al., 1998; Salama et al., 1998), carbohydrate content (Fry et al., 1991 and 1993; Maier et al., 1994), and cold regulated protein synthesis (Gatschet et al., 1996).

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CHAPTER TWO

RELATIONSHIP OF SEASONAL CHANGES IN CARBOHYDRATES AND COLD HARDINESS IN SIX SALTGRASS ACCESSIONS

ABSTRACT

Information is lacking regarding the changes of endogenous soluble carbohydrates of saltgrass [*Distichlis spicata* (L.) Greene] during cold acclimation. The objective of this study was to quantify soluble carbohydrate contents and their relationships to freezing tolerance in six saltgrass accessions, A65, A29 (collected from the Front Range of Colorado), C66, 32 (collected from Nevada), 55 and 48 (collected from South Dakota). The study was carried out on monthly intervals under natural acclimation in two consecutive years (October 1999 – April 2000 and October 2000 – April 2001) at the Horticulture Research Center, Fort Collins, CO. Concurrent with LT_{50} (subfreezing temperature resulting in 50% mortality) data assessment, soluble carbohydrates, including sucrose, fructose, glucose, raffinose and stachyose were measured using gas chromatography (GC). Results indicated significant differences among accessions in LT_{50} and carbohydrate content. Sucrose was the predominant sugar, but did not show clear seasonal trend and had no correlation with freezing tolerance. Fructose, glucose, raffinose and stachyose exhibited clear seasonal changes, showing highest concentrations during mid-winter. In December of both seasons, higher concentration of fructose and

glucose were observed in 48 and 55 than other accessions. Accession A29 had the highest concentration of raffinose in December and January in both seasons. A29 also had the highest stachyose content in midwinter of 1999-2000. Higher fructose, glucose, or raffinose concentrations were frequently observed in accessions of 48, 55, and A29, which coincided with their lowest $LT_{50.}$ In contrast, C66 had the lowest sugar concentrations overall, which related to its sensitivity to lower temperatures. These results indicate that fructose, glucose, raffinose, and stachyose may play important roles in saltgrass freezing tolerance, although this study did not explain the mechanism by which these carbohydrates enhanced freezing tolerance.

INTRODUCTION

Accumulation of carbohydrate in plant tissue is a dynamic system of energy balance. Seasonal variation of carbohydrate reserves differs among grasses. It depends on a balance between photosynthesis and respiration. Thus temperature has marked effects on seasonal variation of carbohydrates, even between day and night. Seasonal changes are characteristic of climatic changes (White, 1973).

Nonstructural carbohydrates (NSC) are thought to serve an important role in freezing tolerance of many plants, including some turfgrasses (Levitt, 1980). Fry et al. (1993) found a positive correlation between sucrose level and surviving stolons in acclimated vs. non-acclimated stolons of centipedgrass [*Eremochloa aphiuroides* (Munro) Hack.]. They suggested that common centipedgrass accumulates sucrose during the fall acclimation period, which contributes to an increase in freezing tolerance of about $2^{\circ}C$ for acclimated vs. non-acclimated plants. Rogers et al. (1975) measured the changes in cold hardiness and carbohydrate composition of zoysiagrass [*Zoysia japonica* (Steud) Meyer] turfgrass during fall and winter. They found an increase in total nonstructural carbohydrate concentration of both rhizome and stolon tissues from September to December and found approximately 15% decrease by March 15. Dionne et al. (2001) assessed the relationship between freezing tolerance of green-type annual bluegrass (*Poa annua* L.) ecotypes and levels of fructans, mono- and disaccharides. Their results indicated a positive correlation between freezing tolerance and the levels of fructans and sucrose. Ball et al. (2002) reported that the concentrations of soluble carbohydrates (sucrose, fructose, glucose and raffinose) increase during cold acclimation, and are important in freezing tolerance of buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.]. Endogenous soluble sugars were also

found to be of importance in freezing tolerance in other species such as grape (*Vitis vinifera* L. Chardonnay and Riesling) (Hamman et al., 1996), *Lonicera carerulea* (L.) (Imanishi et al., 1998), alfalfa (*Medicago sativa* L.) (Castunguay et al., 1995), easter white pine (*Pinus strobes* L.), eastern redcedar (*Juniperus virginiana* L.), Leyland cyperus (x *Cupressocyparis leylandii* Dallim.), Virginia pine (*Pinus virginiana* L.) (Hinesley et al., 1992), red raspberry (*Rubus idaeus* L.) (Palonon, 1999) and aspen (*Populus tremuloides*) (Cox and Stushnoff, 2001).

In contrast, some studies indicated no correlation or relationship between cold hardiness and NSC composition, especially in some grass species with poor freezing tolerance (Bush et al., 2000; Dunn and Nelson, 1974; Fry et al., 1991; Maier et al., 1994). Fry et al. (1991) found little variability in 'floratam' St. Augustinegrass freezing tolerance among sample dates on which a change in starch and sucrose levels was recorded. This indicates no effect of those carbohydrates on freezing tolerance. The results of the work done by Maier et al. (1994) on the same grass supported the results of Fry et al. (1991). Sucrose accumulation did not appear to influence freezing tolerance in stolons of three bermudagrass [*Cynodon dactylon* (L.) pers.] cultivars during fall accumulation (Dunn and Nelson, 1974). Furthermore, no correlation was found between freezing tolerance and NSC in St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze.] and carpetgrass (*Axonopus affinis* Chase.) (Bush et al., 2000). These contrasting observations suggest that the role of NSC in freezing tolerance is not clearly defined and may differ among species.

Most research done on the relationships between freezing tolerance and carbohydrate content has focused on starch and sucrose, especially in warm season

turfgrass. The exception was the study on buffalograss by Ball et al. (2002). Little is known about the presence and the effect of fructose, glucose, and galactose-containing oligosaccharides (raffinose and stachyose) on freezing tolerance in grasses. However, glucose, fructose, raffinose and stachyose were found to be strongly associated with the freezing tolerance of some other species (Castunguay et al., 1995; Cox and Stushnoff, 2001; Hamman et al., 1996; Hinesley et al., 1992; Imanishi et al., 1998; Palonon, 1999; Stushnoff et al., 1993).

In cooperation with a saltgrass breeding project in the Dept. of Horticulture and Landscape Architecture, Colorado State Univ., we studied the freezing tolerance of six saltgrass accessions. The objective of this study was to determine seasonal changes in nonstructural carbohydrates (NSC) levels and relate these changes to the freezing tolerance of the saltgrass accessions.

MATERIALS AND METHODS

Saltgrass accessions used in this experiment were A65, A29 (collected from the Front Range of Colorado), C66, 32 (collected from Nevada), 55 and 48 (collected from South Dakota). These accessions were established in a field nursery at the Horticulture Research Center, Fort Collins, CO. Daily maximum and minimum air temperature were recorded via the cellular-linked weather station located 100 m east of the study area.

Accessions used in this experiment were sampled at monthly intervals from October 1999 through April 2000 and from October 2000 through April 2001. On each sampling date, stolons were collected from each of the two replicated plots for each accession (four replications were sampled in the second season). After washing with cold water to remove soil and plant debris, part of stolons were collected and lyophilized for nonstructural carbohydrates analysis and part of stolons were used to evaluate freezing tolerance as described in Chapter 1.

Analysis of carbohydrates

After freeze-drying (Genesis 25 LL lyophilizer, Virtis, Gardiner, NY), stolons were ground using a Wiley mill, sieved thought an 80-mesh screen, and kept in airtight vials at $-20^{\circ}C$. About 1 mg of ground sample was derivitized using pyridine, hexamethyldisilizane and trimethylchlorsialane to add methyl groups. This results in volatilization of the sugars in the gas chromatography (GC) column. Carbohydrate derivitization was carried out according to Cox and Stushnoff (2001) and Sweely et al. (1963).

1 μL samples were injected into an HP 5890 series II gas chromatograph (Hewlett Packard, Boulder, CO.) with a 30m silica capillary column (J & W DB-1, 0.25 mm inner diameter, 0.25 μm film thickness) and a flame ionization detector. Helium was the carrier gas at a flow rate of 2ml/min. Carbohydrates in the samples were identified by comparing retention times with known standards. Carbohydrates quantifications were determined by comparing peak areas to internal standard area using peak simple 1.72 (SRI, Inc., Torrance, CA).

Data analysis

Data were subjected to analysis of variance to test the effect of accession, sampling date, year, and their interactions using the GLM procedure (SAS Institute, 1991). The two seasons of freezing tolerance and carbohydrate data were presented separately because of the significant season effect. Monthly means were separated by Fisher's protected least square difference (LSD) at $P \leq 0.05$. Pearson's correlation coefficient between individual carbohydrate and LT_{50} were analyzed using the CORR procedure of SAS for each season. Regression analysis using the REG procedure of SAS were done to test the association between LT_{50} as independent variable and carbohydrate concentrations as dependent variable for both seasons.

RESULTS

Freezing tolerance

Freezing tolerance results were presented in Chapter 1 and summarized again in respect to the minimum and maximum air temperatures that prevailed at the time of sampling in Figures 2.1 and 2.2.

Soluble carbohydrates

Overall both seasons. Sucrose was the most predominant sugar but had no relationship with freezing tolerance. Fructose and glucose followed sucrose in abundance and were closely correlated with freezing tolerance. Although stachyose and raffinose were present in low concentrations in all accessions, they seemed to have a good correlation with freezing tolerance (Fig. 2.3). Concentrations of soluble carbohydrates varied significantly with sampling time, accession and their interactions (Table 1.1). Fructose, glucose and raffinose exhibited a clear trend of seasonal changes (Table 2.2). Stachyose also exhibited a clear trend of seasonal changes and was completely absent in October and April, with only low concentrations in midwinter months.

Oct. 1999 – April 2000. Fructose, glucose, raffinose and stachyose increased from October to December and January. Fructose, glucose and stachyose reached their maximum in December. Raffinose reached its maximum in January (Fig. 2.3). The highest fructose content was observed in accession 48 in December [34.8 μ mol/g dry weight (DW)]. Accession 55 had the highest glucose content in December too (34.4 μ mol/g DW). A29 had the highest raffinose concentration in January (1.6 μ mol/g DW). Stachyose highest concentration was in A29 (0.4 μ mol/g DW) in December and
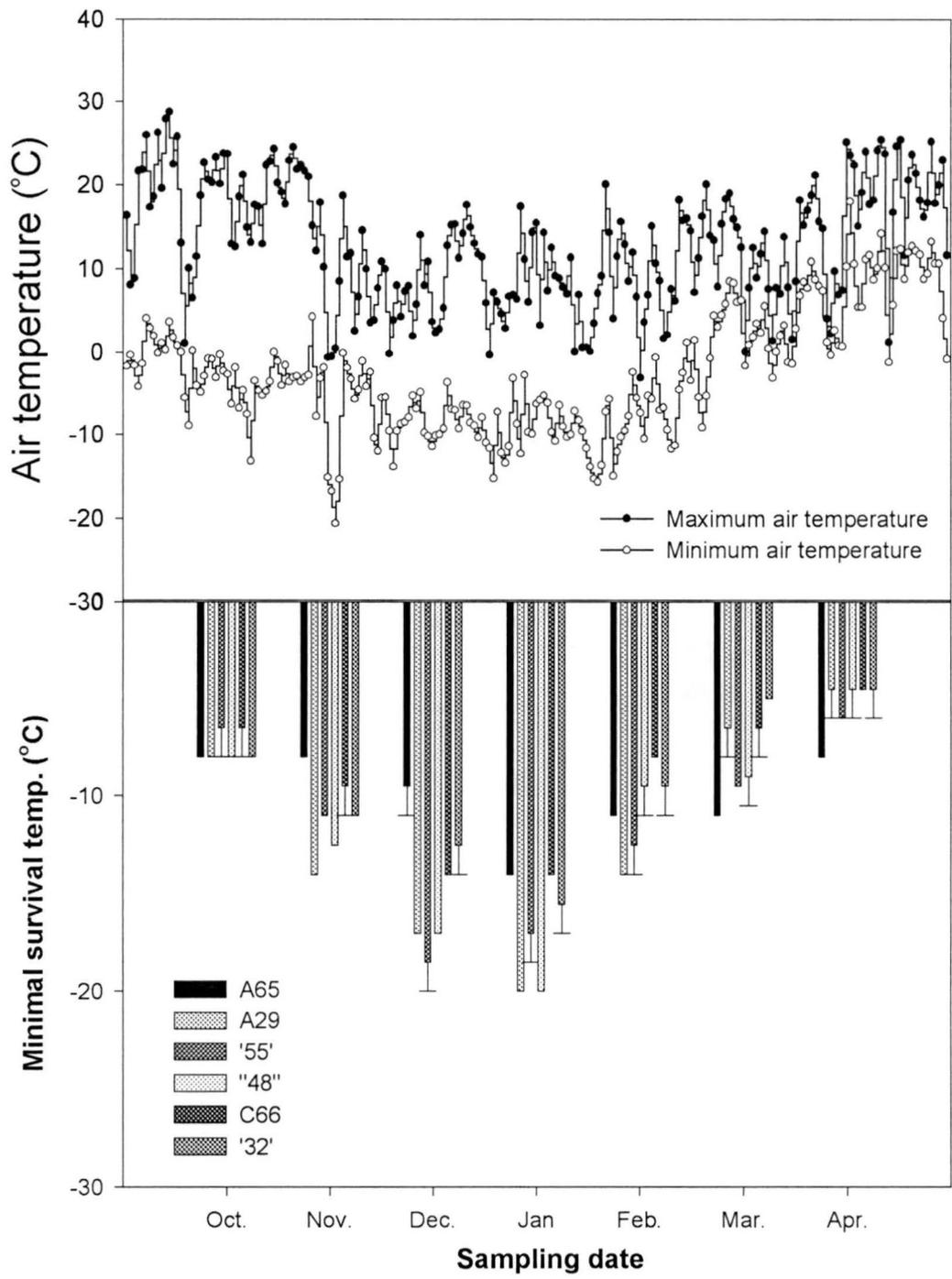
Fig. 2.1. Seasonal patterns of minimal survival temperatures of six saltgrass accessions sampled from Oct. 1999 to April 2000 in relation to the prevailing minimum and maximum air temperature at the sampling dates.
Bars represent the standard errors.
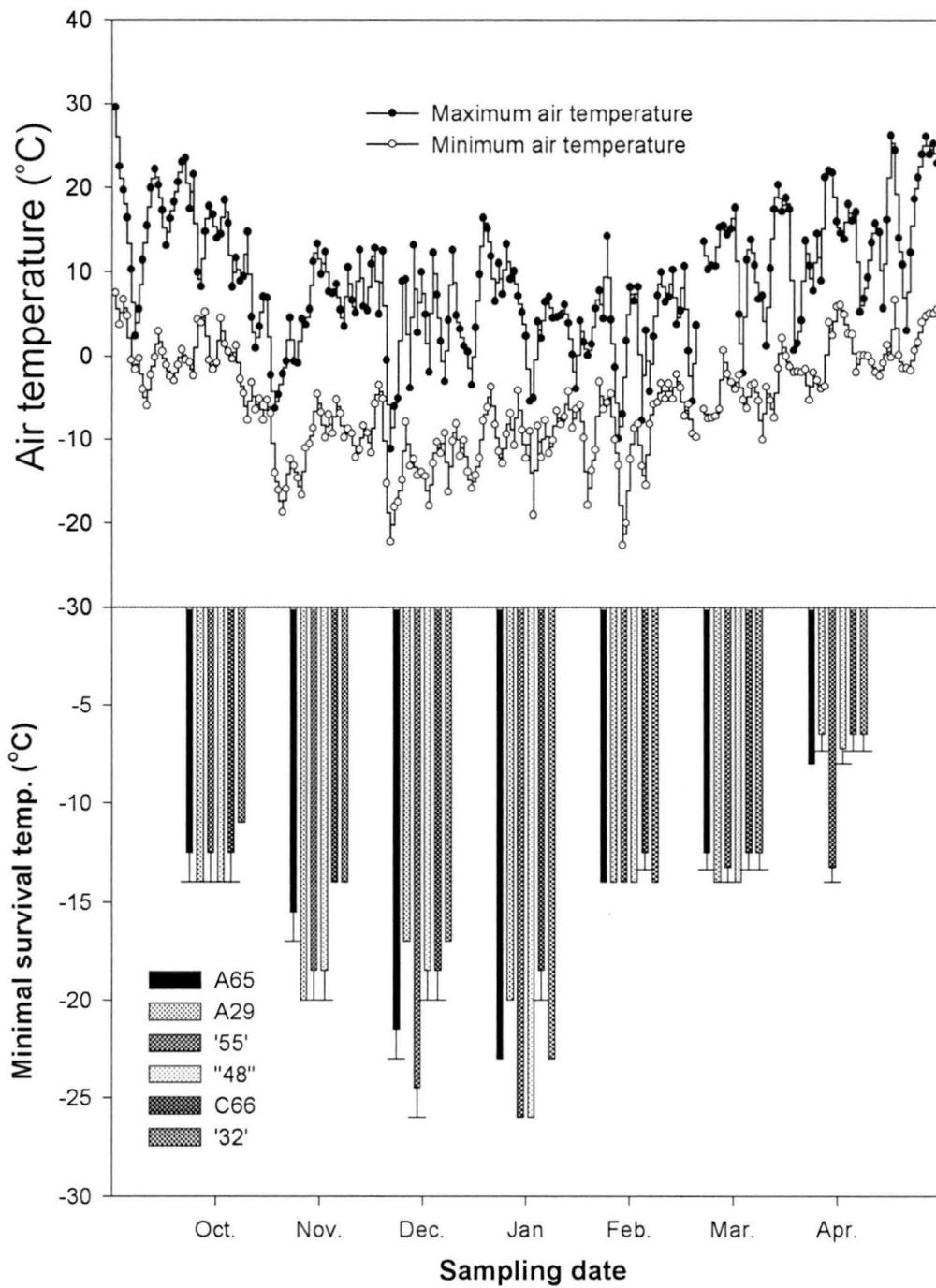
Fig. 2.2. Seasonal patterns of minimal survival temperatures of six saltgrass accessions sampled from Oct. 2000 to April 2001 in relation to the prevailing minimum and maximum air temperature at the sampling dates.
Bars represent the standard errors.

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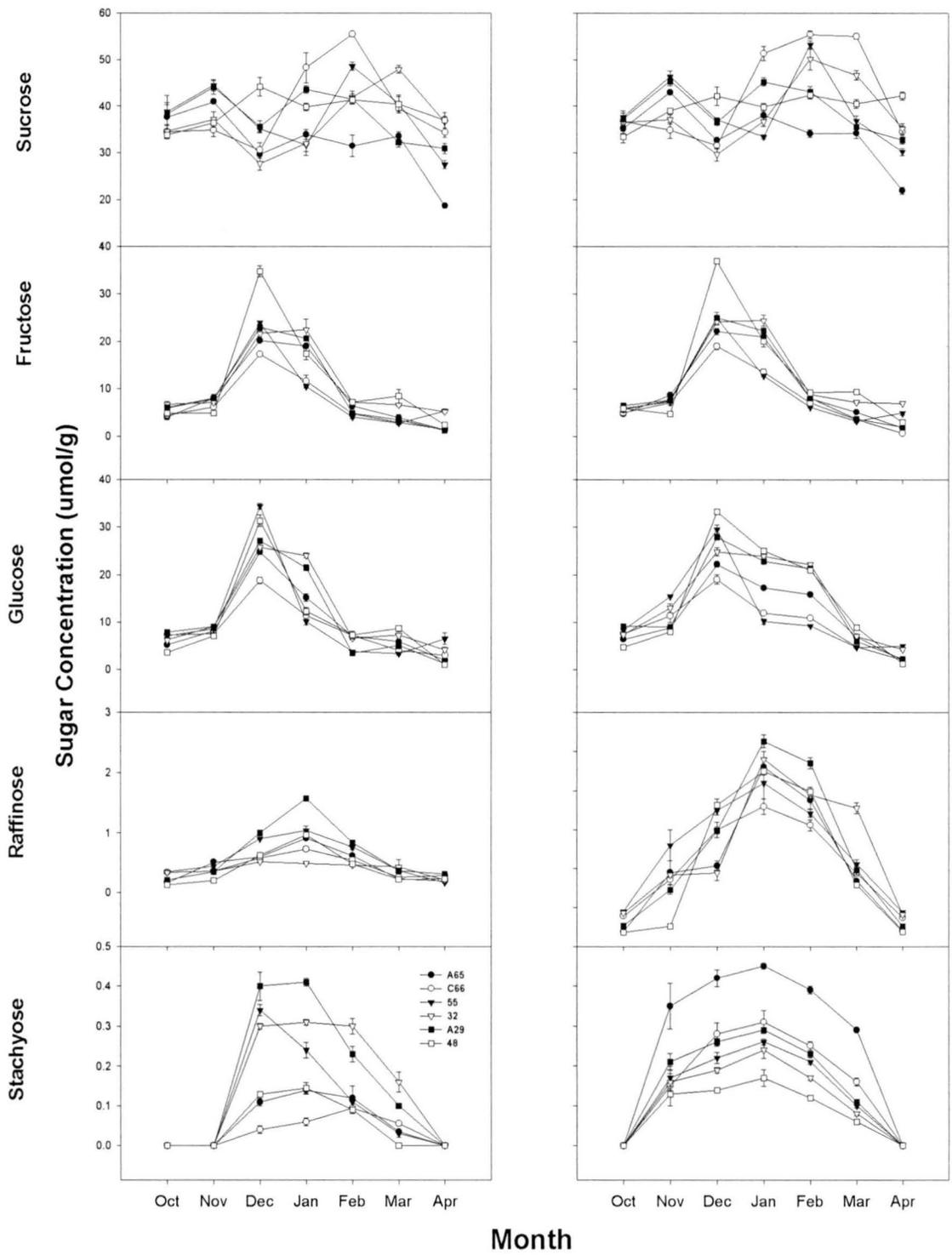
Fig. 2.3. Mean contents of sucrose, fructose, glucose, raffinose and stachyose of six saltgrass accessions tested from October 1999 to April 2000 (left panel) and from October 2000 to April 2001 (right panel). Bars represent the standard errors.

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Table 2.1. ANOVA with mean squares and treatment significance of LT_{50}, sucrose, fructose, glucose, raffinose, and stachyose of six saltgrass accessions from October to April 1999-2000 and 2000-2001 winter seasons in Fort Collins, CO.

Source	Mean square					
	LT_{50}	Sucrose	Fructose	Glucose	Raffinose	Stachyose
1999-2000						
Accessions	23.0[*]	98.1[*]	37.8[*]	18.0[*]	0.12[*]	0.02[*]
Month	198.7[*]	204.1[*]	709.2[*]	848.4[*]	0.77[*]	0.08[*]
Accessions x month	8.3[*]	66.8[*]	18.1[*]	22.7[*]	0.14[*]	0.02[*]
2000-2001						
Accessions	34.8[*]	139.2[*]	55.9[*]	75.4[*]	0.2[*]	0.07[*]
Month	378.7[*]	460.9[*]	956.3[*]	1074.8[*]	9.3[*]	0.22[*]
Accessions x month	8.5[*]	98.0[*]	19.9[*]	36.1[*]	0.2[*]	0.01[*]

[*] Significant at $P < 0.0001$.

Table 2.2. Rate of change (%) in the concentrations of fructose (F), glucose (G) and raffinose (R) from Oct. to Dec. and from Dec. to April through 1999-2000 and 2000-2001 winter seasons in Fort Collins, CO. in six saltgrass accessions.

Accessions		A65			C66			55			32			A29			48		
Sugars		F	G	R	F	G	R	F	G	R	F	G	R	F	G	R	F	G	R
	Change (%)																		
First season (99 – 00)	Oct. - Dec.	400	379	259	297	157	66	305	386	157	222	315	49	281	246	376	606	767	392
	Dec. – April	- 93	- 92	- 69	- 91	- 84	- 51	- 78	- 81	- 81	- 76	- 84	- 60	- 94	- 95	- 69	- 93	-97	- 64
Second season (00 – 01)	Oct. - Dec.	367	246	1111	279	151	362	322	253	289	324	234	121	283	203	452	524	606	911
	Dec. - April	- 91	- 91	- 91	- 96	- 89	- 79	- 80	- 83	- 75	- 71	- 83	- 57	- 92	- 92	- 83	- 92	- 96	- 90

January. All sugar concentrations declined gradually from January to April approaching zero for stachyose in April in all accession and 0.01 μ mol/g DW for raffinose.Fructose reached its lowest concentration in April in A65, C66 and A29 ($\approx$ 1.4 μ mol/g DW). 48 had the lowest glucose concentration in April (1.0 μ mol/g DW).

Oct. 2000 – April 2001. All sugars in the second season followed a similar trend as in the first season. Fructose concentration was 37 μ mol/g DW in December in 48 with an increase of 2.2 μ mol/g DW over its concentration in the first season. In contrast, 55 exhibited a lower glucose concentration than in the first season (29.5 μ mol/g DW). Accession 48 had the highest glucose concentration with 33.2 μ mol/g DW in December. A29 exhibited the greatest raffinose concentration (2.6 μ mol/g DW) with a 1.0 μ mol/g DW increase as compared to the first season in January. Stachyose was relatively higher in A65 (0.45 μ mol/g DW) in January in contrast to the first season when A29 had the highest stachyose concentration.

Correlation of carbohydrate content and freezing tolerance. Sucrose demonstrated no correlation with LT_{50} in both seasons (Table 2.3). In the first season, correlations between LT_{50} vs. fructose, glucose, raffinose and stachyose were significant with linear coefficients of − 0.71, - 0.67, - 0.56 and − 0.59 respectively. The correlations between LT_{50} vs. fructose, glucose, raffinose and stachyose were significant also in the second season with linear correlation coefficients of − 0.7, - 0.67, - 0.61 and − 0.61 respectively. Generally, fructose had the highest correlation with LT_{50} in both seasons followed by glucose, stachyose and raffinose.

Regression of LT_{50} as independent variable with sugars concentrations as dependent variable showed a significant association between LT_{50} and fructose, glucose, raffinose,

and stachyose while no association was found between LT_{50} and sucrose concentration
(Fig. 2.4).

Table 2.3. Correlation coefficients between freezing tolerance (LT_{50}) and individual soluble carbohydrate content in the stolons of the tested accessions (A65, A29, 55, 48, C66 and 32) of saltgrass sampled from October 1999 to April 2000 and from October 2000 to April 2001. A negative coefficient indicates that the lower LT_{50} is associated with an increase in carbohydrate content.

Parameter	Sucrose	Fructose	Glucose	Raffinose	Stachyose
1999-2000					
Lt_{50}	-0.11^{NS}	-0.71^{***}	-0.67^{***}	-0.56^{***}	-0.59^{***}
Sucrose		-0.01^{NS}	-0.07^{NS}	0.33^{**}	0.22^{*}
Fructose			0.95^{***}	0.49^{***}	0.50^{***}
Glucose				0.44^{***}	0.51^{***}
Raffinose					0.87^{***}
2000-2001					
Lt_{50}	-0.11^{NS}	-0.7^{***}	-0.67^{***}	-0.61^{***}	-0.61^{***}
Sucrose		0.02^{NS}	0.16^{NS}	0.43^{***}	0.25^{**}
Fructose			0.85^{***}	0.56^{***}	0.50^{***}
Glucose				0.74^{***}	0.56^{***}
Raffinose					0.68^{***}

$^{NS, *, **, ***}$ Nonsignificant or significant at $P \leq 0.05$, ≤ 0.005, and < 0.0001, respectively.
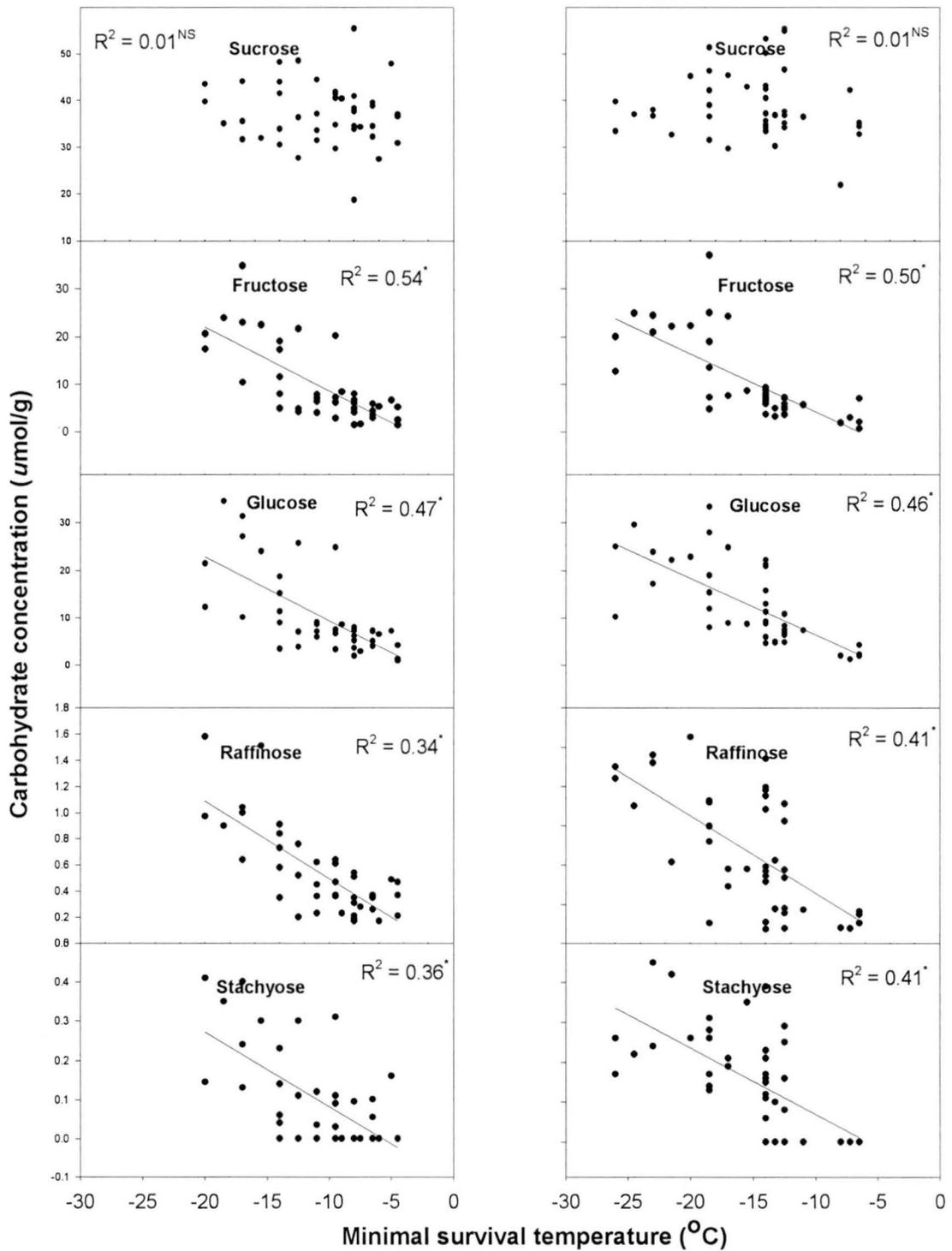
Fig. 2.4. Relationship between carbohydrates content and minimal survival temperatures of six saltgrass accessions sampled from Oct. 1999 to April 2000 (left panel) and from October 2000 to April 2001 (right panel). $^{NS, *}$ Nonsignificant or significant at $P < 0.0001$.

DISCUSSION

The climatic conditions were significantly different during the 1999-2000 and 2000-2001 seasons (Figures 2.1 and 2.2). The second season had relatively cooler air temperatures than the first season, which may have contributed to the higher freezing tolerance in the second season. The variation among the accessions within the same season may be associated with the origin of each accession.

In both seasons, there was no significant difference in freezing tolerance among all accessions in October as tissues were not acclimated. Carbohydrate concentrations followed the same pattern in this month with low concentrations. All sugars except for sucrose exhibited an increase from October to December and January, and decreased gradually from January to April, following a similar pattern of LT_{50}.

Sucrose concentration was up and down showing no clear trend with seasonal changes. Fry et al. (1993) suggested that sucrose may play a role in the freezing tolerance of Oklawn centipedgrass stolons. Dionne et al. (2001) also noticed that an increase in sucrose levels in crowns of annual bluegrass coincided with its maximum freezing tolerance. This does not appear to occur in saltgrass accessions. Higher fructose, glucose, or raffinose concentrations were frequently observed in accessions 48, 55, and A29, which coincided with their better freezing tolerance (i.e., lower LT_{50}) when compared with other accessions. In contrast, C66 had the lowest sugar concentrations overall, which related to its sensitivity to lower temperatures. These results indicate that fructose, glucose, and raffinose play important roles in saltgrass freezing tolerance. The significant linear relationships of LT_{50} vs. fructose, glucose, raffinose, and stachyose concentrations support this conclusion (Fig. 2.4).

Imanishi (1998) suggested that raffinose and stachyose may play a role in the freezing tolerance of the shoot apices of *Lonicera caerulea* L. var. *emphyllocaryx* Nakai. Castonguay et al. (1995) related variations in alfalfa freezing tolerance to differences in sucrose, raffinose and stachyose concentrations of overwintering crowns. Hinesley et. al. (1992) related cold hardiness of coniferous trees to raffinose and sucrose. Most of these studies stressed the importance of carbohydrates in freezing tolerance but no study described the mechanism or the physiological basis of this action. Nevertheless, it is the general belief that the presence of carbohydrates as solutes in high levels with the decrease in temperature helps in increasing the osmotic pressure, which decreases freezing point and enhances freezing tolerance. Generally we can consider carbohydrates as cryoprotectants as they prevent ice formation and cell desiccation.

Accession C66, the most cold tender accession, was originally collected from Nevada. This area is relatively warm so that it may be genetically adapted to warm climates and carbohydrate concentrations responded to that adaptation. Accession 32 was also collected from Nevada but it had reasonable sugar concentrations with associated freezing tolerance. The superiority of 32 over C66 in freezing tolerance and carbohydrate content might be related to higher adaptive ability of 32 for cold climates.

Correlation analysis indicated a close interrelation between fructose and glucose (r = 0.95 in the first season and 0.85 in the second season). This is expected since these hexoses are the intermediates of sucrose cleavage, which then enters the metabolic pathways of the cell to provide the energy and substrates required for viability and growth. It is well known that sucrose hydrolyzes into fructose and glucose by the catalytic action of invertase isoenzymes or converts into uridine diphosphate-glucose

(UDP-glucose) and fructose by sucrose synthase (Avigad and Dey, 1997). Raffinose and stachyose were found to be highly correlated also (r = 0.87 in the first season and 0.68 in the second). Stachyose coexists with raffinose although it is synthesized at the expense of the pool of raffinose and galactinol (Avigad and Dey, 1997). Kandler and Hopf (1980) suggested a seasonal variation in stachyose and related oligosaccharides that enhances frost tolerance in winter hardy plants. There was also a significant correlation between sucrose and raffinose (r = 0.33 in the first season and 0.43 in the second season), which can be explained by their shared metabolic pathway during synthesis (Avigad and Dey, 1997).

In summary, our results suggest that 55, 48 and A29 had the highest sugar concentrations overall, which were associated with their greater freezing tolerance. C66 had low carbohydrate concentrations and correspondingly poor freezing tolerance. Sucrose was the predominant carbohydrate but, had no correlation with freezing tolerance. Fructose and glucose followed sucrose in abundance and correlated well with freezing tolerance. Raffinose and stachyose concentrations were the lowest but had a significant correlation with freezing tolerance. Our study was quantitative and qualitative and did not explain the mechanism by which carbohydrate concentration can enhance freezing tolerance. Further research is needed to address this issue.

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CHAPTER THREE

EFFECT OF NITROGEN LEVEL ON ESTABLISHMENT, PRODUCTION AND NUTRIENT CONTENT OF SALTGRASS

ABSTRACT

The vegetation cover of the earth depends on inorganic nitrogen for establishment and growth. The efficient utilization of nitrogen in grass production is essential to reduce the risk of water and air pollution in addition to minimizing production expenses. Limited information is available regarding the effect of nitrogen on saltgrass [*Distichlis spicata* (L.) Greene] establishment, productivity and nutritive value and its potential as a range species. The objectives of this study were to: (a) determine nitrogen requirements for two saltgrass accessions during establishment and growth; (b) evaluate nutrient content of those accessions as related to nitrogen fertilizer levels; and (c) evaluate saltgrass as a rangeland species. The study was carried out at the Horticulture Research Center, Fort Collins, CO. Saltgrass accessions A24 and A138, collected from the Front Range of Colorado, were established from stolon plugs in the field in June 2000 and again in 2001 with four replications. Within each saltgrass plot, nitrogen treatments (0, 150, 300, 450, 600 kg N/ha) in the form of urea (46 %) were applied as sub-plots. Canopy cover (%) was monitored monthly, productivity (kg/ha) was assessed at the end of each growing season, and essential mineral elements (Ca, P, K, Na, S, Mg, Fe, Zn, Cu, Mo, Mn, and Co) (g/kg) were analyzed using Inductively Coupled Plasma (ICP). Total

nitrogen (g/kg) was measured using a combustion furnace method (LECO CHN-1000). Results indicated positive linear relationships between percentage of cover, productivity, tissue nitrogen and protein contents and applied nitrogen levels in both seasons. Ca, P and Fe had a positive association while Na, S and Mg had a negative association with nitrogen levels. Establishment in terms of cover and productivity, and nutritive value of the two tested saltgrass accessions increased with increasing N fertilization rate. However, nitrogen had no effect during the first month of establishment on cover when water was critical. The rate of 450 kg/ha is recommended for the best cover percentage. Also, we recommend the use of saltgrass to improve pastures in saline areas, and other areas where potable water is limited, because of its nutritive value.

INTRODUCTION

The vegetation cover of the earth depends on inorganic nitrogen for establishment and growth. The efficient utilization of nitrogen in grass production is essential to reduce the risks of water and air pollution as well as production expenses. Fertilization studies have been conducted on native rangelands in the Great Plains (Berg, 1990; Gillen and Berg, 1998; Gillen et al., 1987; Hart et al., 1995; Samuel et al., 1980). Nitrogen fertilization has been shown to increase forage dry matter and crude protein concentrations of bermudagrass [*Cynodon dactylon* (L.) pers.] (Burton et al., 1956) and wheatgrass (*Agropyron spp.*) (Cook, 1965). In a controlled greenhouse environment at Colorado State Univ. (fall of 1988) with supplemental lighting for 15 hours, Richard and Redente (1995) carried out an experiment to determine if the interaction of competition and nutrient availability affects relative growth and nutrient acquisition of blue grama [*Bouteloua gracilis* (H. B. K.) Lag.] and buffalograss [*Buchloe dactyloides* (Nutt.) Engelman]. They found an increase in both shoot and root biomass of blue grama in response to N and P addition, which suggested that both nutrients were limiting. They also noticed the same behavior in the second harvest of buffalograss. They recorded increases in nutrient content of both species by the second harvest.

Read and Hipp (1998) examined the nitrogen and phosphorus fertilizer requirements of tall fescue (*Festuca arundinacea* Schreb) grown on Houston blackland prairie soils at the Texas A&M University Research and Extension Center at Dallas. They found an increase in dry matter with increasing nitrogen rate. They also found an increase in protein level with increased N. They could not detect any differences in dry matter production, seed production, or protein content with P treatments. Bregard et al. (2001)

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studied the relationship between dry matter yield, nutritive value and biomass partitioning under limiting and nonlimiting nitrogen rates in timothy (*Phleum pratense* L.). They found a significant nitrogen effect on dry matter, forage yield and forage nitrogen concentration. Increases in forage dry matter yield and crude protein were similarly reported in buffalograss due to nitrogen fertilization (Springer and Taliaferro, 2001). Munshaw et al. (2001) found a significant positive linear relationship between stolon production and urea nitrogen fertilizer in bermudagrass as well. In a 3-year field study, Oral and Acikgoz (2001) studied the influence of nitrogen application timing, 30g m^{-2} year^{-1}, applied annually with different regimes (single spring, single fall, spring + fall, spring + fall + summer and monthly from April through September) under irrigated conditions on growth and quality of a turfgrass mixture of ryegrass (*Lolium pereme* L.), Kentucky bluegrass (*Poa pratensis* L.), creeping red fescue (*Festuca rubra* var. rubra L.) and chewings fescue (*Festuca rubra* var. commutata Guad.). Positive correlations were found between N fertilizer rate and application timing with color, turf quality, clipping yield and shoot density. Bittman and Kowalenko (1998) conducted a similar study to determine the effect of rate and distributions (at the start of grass growth in early spring, and immediately after cuts 1, 2 and 3 with a proportion of 1.00/0/0/0, 0.50/0.25/0.25/0 and 0.25/0.25/0.25/0.25) of N on whole-season herbage yield and quality. Their results indicated an increase in whole-season yield by increasing N rate. Distribution of N uniformly through the season resulted in a 5% increase compared with applying all of the nitrogen at the beginning of the season. However, applying the entire N amount at the beginning of the season increased herbage crude protein.

The productivity of North America salt marshes is limited by the availability of nitrogen (Gallagher, 1975; Squiers and Good, 1974; Valiela and Teal, 1974). The only study conducted on nitrogen influence on saltgrass was by Smart and Barko (1980) where they grew saltgrass on saline sediments. They thus had an interaction of salinity with nitrogen effect and as a result their conclusion had to consider sediments salinity in addition to the positive effect of nitrogen.

Essential minerals are classified into major and trace elements depending upon their concentrations in the animal body. All essential mineral elements are believed to have one or more catalytic functions in the cell. It thus is very important to know the concentration of those elements in addition to N and crude protein in saltgrass as parameters to evaluate it as a rangeland species or as a fodder.

No published study has evaluated saltgrass as a rangeland grass. Some reports have consider saltgrass an important forage species, especially in the saline-alkaline basins of the western United States (Cluff et al., 1983) as well as many of the salt marsh areas of the interior United States and similar areas where potable water is of great demand. We evaluated saltgrass as a potential new rangeland species which can fill the gap in areas where other species can not succeed. It is an important species for wildlife, especially in the diet of waterfowl and the Florida salt marsh vole (*Microtus pennsylvaicus duckecampbelli*) which is on the Endangered and Threatened Species List of Southern United States. Ducks eat the dried seeds and wild geese eat the tender forages. It is also significant in the salt marshes, which provide nesting grounds for birds, fish and larvae of many species of marine invertebrate animals. As salt marsh plants decompose, their

stored nutrients provide a steady source of food for clams, crabs, and fish (USDA, NRCS, National Plant Data Center).

Distichlis spicata (L.) Green (saltgrass) is a widely distributed, rhizomatous, perennial grass common in the Atlantic coast tidal areas as well as inland saline marshes of many regions of North America including Mexico (Hansen et al., 1976). Because of the vigorous rhizome growth, and asexual reproduction, saltgrass is one of the most versatile plants in the saltgrass meadow. It can directly invade an existing stand of vegetation by sending out rhizomes. It is also a potential species for revegetation of mine spoils and roadsides in the semiarid west (Pavlicek et al., 1977).

Saltgrass meadows are found in many climatic zones within the western United States and western Canada. Precipitation in its habitat ranges from 15 cm in Escalante, Utah to 66 cm in Oklahoma. Temperature ranges from -10° C to 28° C. The number of frost-free days varies from 90 days in the northern areas to 207 days in the southern areas of the United States. The presence of saltgrass usually indicates a clayey soil, good moisture, and high levels of salts. *Distichlis spicata* is one of the most salt tolerant graminoides frequently associated with salt marshes and inland saline wetlands, and its response to soil salinity has been the subject of a number of growth-chamber and greenhouse investigations (Adams, 1963; Hansen et al., 1976; Kemp and Cunningham, 1981; Parrondo et al., 1978; Smart and Barko, 1980; Taylor, 1938; Tiku, 1976; Warren and Brockelman, 1989). The objectives of this study were to determine (a) nitrogen requirements for two saltgrass accessions during establishment and growth; (b) how nutrients content of those accessions is related to nitrogen fertilizer levels, and (c) to evaluate saltgrass as a rangeland species.

MATERIALS AND METHODS

Saltgrass accessions chosen for this experiment were A24 and A138 (collected from the Front Range of Colorado). A24 showed a relatively higher growth rate than A138 under similar field conditions. Stolons were collected from a field nursery at Horticulture Research Center, Colorado State University, Fort Collins, CO. to initiate plugs of equal sizes in the greenhouse at approximately 25°C. Irrigation was applied manually to provide about 3-5 mm/day. Those plugs were transplanted to field plots in June of 2000 and 2001. Daily maximum and minimum air temperature and precipitation were recorded via the cellular-linked weather station located 100 m east of the study area.

Field plots were laid out in a split plot design with accessions as main plots and nitrogen levels as sub-plots. Between main plots trenches of 3 feet deep were made to prevent stolon invasion. Soil samples were collected from sub-plots and analyzed for nitrogen content before applying nitrogen treatments. No significant difference was noted among them. Soil pH ranges from 8.2 to 8.4 and irrigation water pH ranges from 7.5 to 8.2. Treatments were control (0.00), 150, 300, 450 and 600 kg N/ha as urea (46-0-0) divided equally for the 3 applications in June, July and August.

Cover percentage was measured monthly by calculating the covered area (covered area was measured for each plug as a circle after measuring the average diameter) in relation to the total plot area. Aboveground growth was harvested at the end of each growing season, oven dried at 70 ° C to constant weight and weighted for determination of dry matter. Plugs productivity was calculated in relation to total plot area, so it is not representing saltgrass productivity in closed stands but used only to compare treatment effect. Portions of the dried matter were ground in a Wiley mill, sieved through a 60-

mesh screen, and kept in airtight vials for nitrogen and mineral analysis. One gram of powdered plant material was digested using nitric and perchloric acids for Ca, P, K, Na, S, Mg, Fe, Zn, Cu, Mo, Mn, and Co analysis using Inductively- Coupled Plasma atomic emission spectrophotometry (ICP-AES) (Model 975 plasma Atomcomp, Thermo Jarrell Ash Corp., Franklin, MA 02038). Total nitrogen was measured using the combustion furnace (Model CHN-1000 elemental analyzer, LECO Corporation, St Joseph, Michigan). Crude protein was calculated by multiplying total nitrogen by the factor 6.25 (Oelberg, 1956; Pirie, 1955; Shahba, 1994).

Data analysis:

All measured parameters (cover, productivity, elements, nitrogen and protein) were subjected to analysis of variance (ANOVA) procedure of SAS (SAS Institute, 1989) incorporating seasons, nitrogen levels and accessions. Means separation among accessions and nitrogen levels were performed at $P = 0.05$ by Fisher's LSD test. Regression analysis using the REG procedure of SAS was done to test the association between nitrogen level as independent variable and each element, cover, productivity, N, and protein as dependent variables for both seasons.

RESULTS

Cover percentage.

Cover percentage increased gradually from the first month to the third month in both seasons (Fig. 3.1). There was no treatment effect during the first month. Treatment effect was evident from the second month in a linear manner. By the third month, N effect on grass cover reached a plateau and there was no significant difference between nitrogen levels at 450 and 600 kg/ha. The maximum coverage was 6.2% in the first season and 6.4% in the second season with a nitrogen level of 450 kg/ha. Over the two seasons, A138 showed a relatively higher cover percentage through all months than A24. However, there was no significant statistical difference. Regression analysis showed a good association between nitrogen rate and cover percentage (R^2 = 0.94 for first season and 0.95 for the second).

Productivity.

Since there was a significant difference in total productivity between the two seasons, data on productivity is presented by season. A typical linear relationship was found between productivity and nitrogen levels in the first season. A24 had a greater production than A138 at all nitrogen levels (Fig. 3.2). There was a significant difference between accessions and among nitrogen levels but the accession x N interaction was not significant. The LSD test clearly indicated that A24 had greater production with a mean of 181.9 kg/ha. The mean production of A138 was 129.5 kg/ha. Nitrogen level of 600 kg/ha achieved the greatest productivity in both accessions (241.9 kg/ha in A24 and 186.5
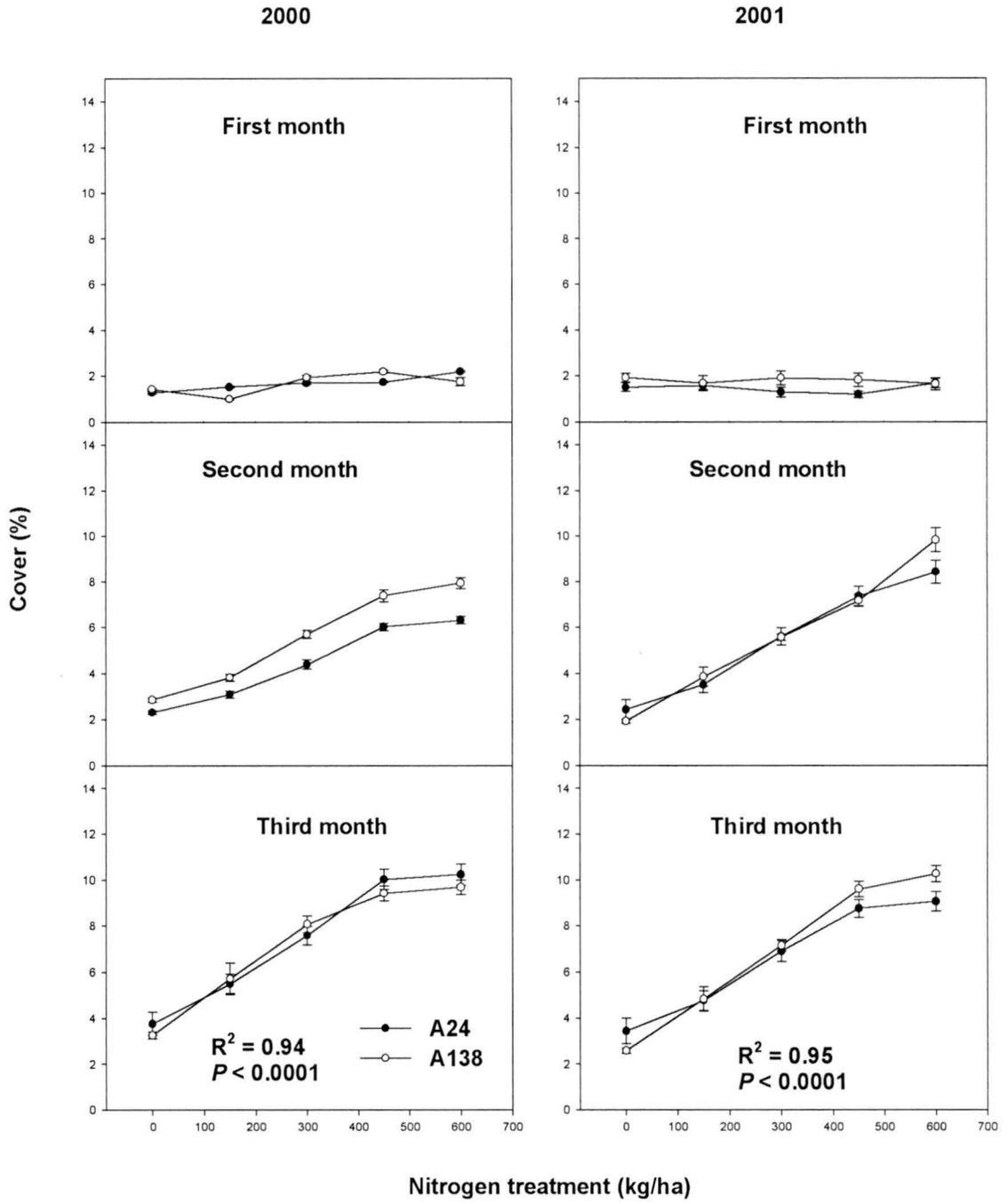
Fig. 3.1. Cover of tested saltgrass accessions in response to different nitrogen treatments in the first season (left panel) and in the second season (right panel). Bars represent the standard error.
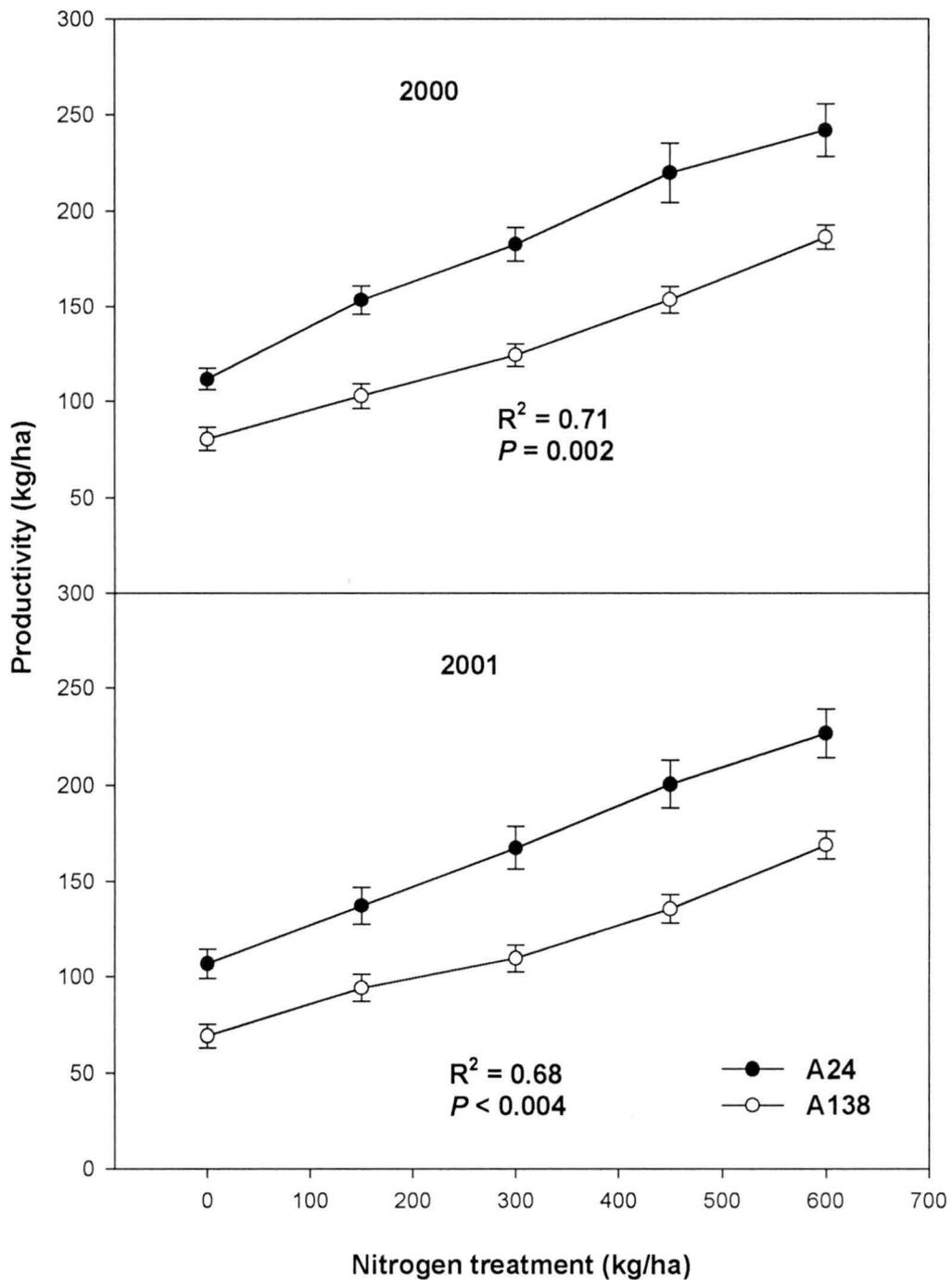
Fig. 3.2. Total productivity of tested accessions in response to different nitrogen treatments in the two seasons of the study. Bars represent the standard errors.

kg/ha in A138). Regression analysis indicated a significant association between productivity and nitrogen levels ($R^2 = 0.71$).

In the second season, there was also a linear relationship between productivity and nitrogen levels. Productivity of this season was less than that of the first season (Fig. 3.2). A24 achieved greater productivity with a mean of 167.7 kg/ha than A138, which had a mean production of 145.4 kg/ha. Analysis of variance indicated a significant difference between the two accessions and among the nitrogen levels. The level of 600 kg/ha achieved the highest production in A24 (226.6 kg/ha) and in A138 (169.2 kg/ha). Regression analysis indicated a significant association between productivity and nitrogen levels ($R^2 = 0.68$).

Nitrogen and protein contents.

Significant differences between the two seasons were found in nitrogen and protein contents. Similar linear relationships between nitrogen and protein content and nitrogen levels were found in both seasons (Fig. 3.3 and 3.4).

In the first season, there was a significant difference between accessions in nitrogen and protein contents. A138 had higher mean nitrogen (16.3 kg/ha) and consequently greater mean protein content (101.7 kg/ha) than those of A24, which had a mean nitrogen content of 12.5 kg/ha, and a mean protein content of 78.2 kg/ha. Regression analysis showed a significant association between nitrogen and protein content and nitrogen levels ($R^2 = 0.81$).

In the second season, similar linear relationships were found with an increase in nitrogen and protein content. A138 had the higher mean N (18.9 kg/ha) and protein
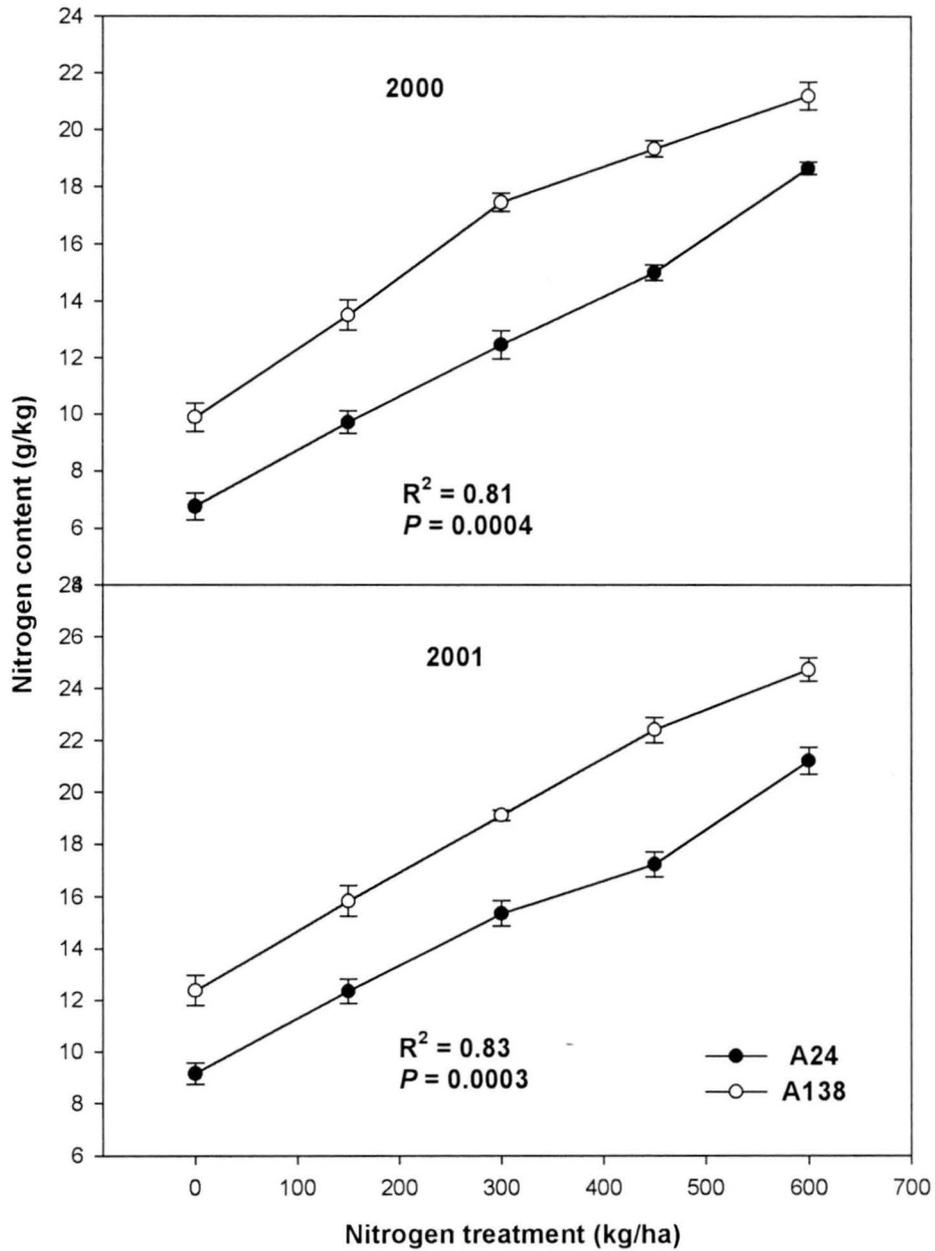
Fig. 3.3. Nitrogen content of tested accessions in response to different nitrogen treatments in the two seasons of the study. Bars represent the standard errors.
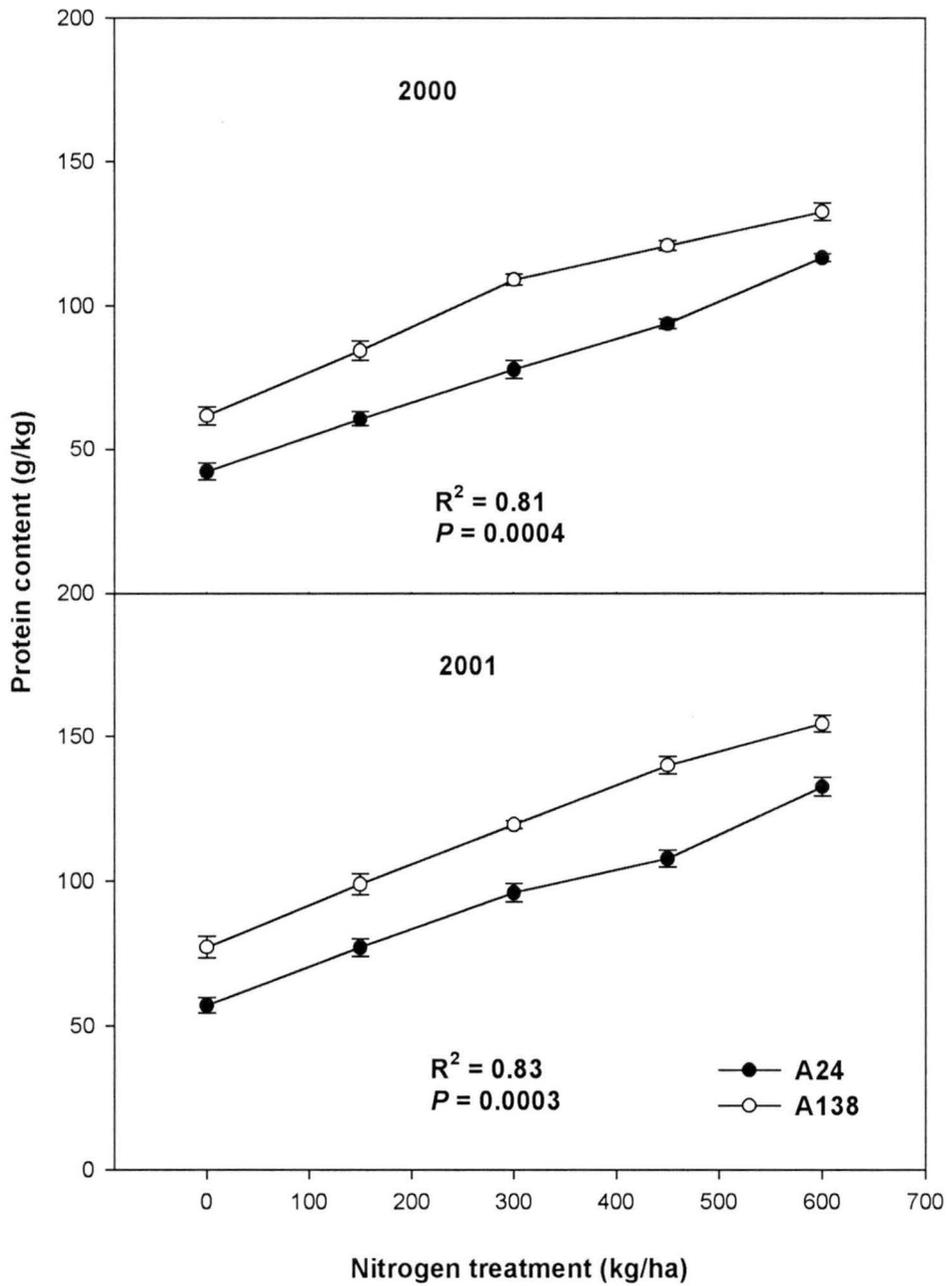
Fig. 3.4. Protein content of tested accessions in response to different nitrogen treatments in the two seasons of the study. Bars represent the standard errors.

(118.0 kg/ha) than A24, which had a mean of 15.1 kg/ha nitrogen and 94.1 kg/ha protein. Regression analysis indicated higher association than that of the first season ($R^2 = 0.83$).

Major elements.

Major elements from the animal nutrition point of view are defined as minerals present in high concentration in the animal body and are believed to have one or more catalytic functions in the animal cell (McDonald et al., 1988). The quantified major elements in tested accessions were Ca, P, K, Na, S, and Mn. Analysis of variance indicated a significant difference in major minerals contents between the two seasons.

In the first season, Ca was the predominant element in both A24 and A138 with a mean of 7.0 g/kg in A24 and 6.5 g/kg in A138 (Fig 3.5). Ca content had a linear relationship with nitrogen levels and a significant positive association ($R^2 = 0.94$). K followed Ca in dominance with a mean content of 4.04 g/kg in A24 and 3.93 g/kg in A138 with no significant statistical difference between them. Also, K content showed no association with nitrogen levels. Mg content had a significant difference between the two accessions with a mean of 2.92 g/kg in A24 and 2.59 g/kg in A138. There was a negative association between Mg content and nitrogen levels ($R^2 = -0.77$). S had a significant difference between the tested accessions with a mean content of 1.58 g/kg in A24 and 1.87 g/kg in A138. A negative association was also found between S content and nitrogen levels ($R^2 = -0.65$). P content was little in both accessions with a significant statistical difference and a positive association with nitrogen levels ($R^2 = 0.85$). Na was the lowest among all major elements and there was no significant difference between the accession with a mean of 0.48 g/kg in A24 and 0.53 g/kg in A138. There was a negative association
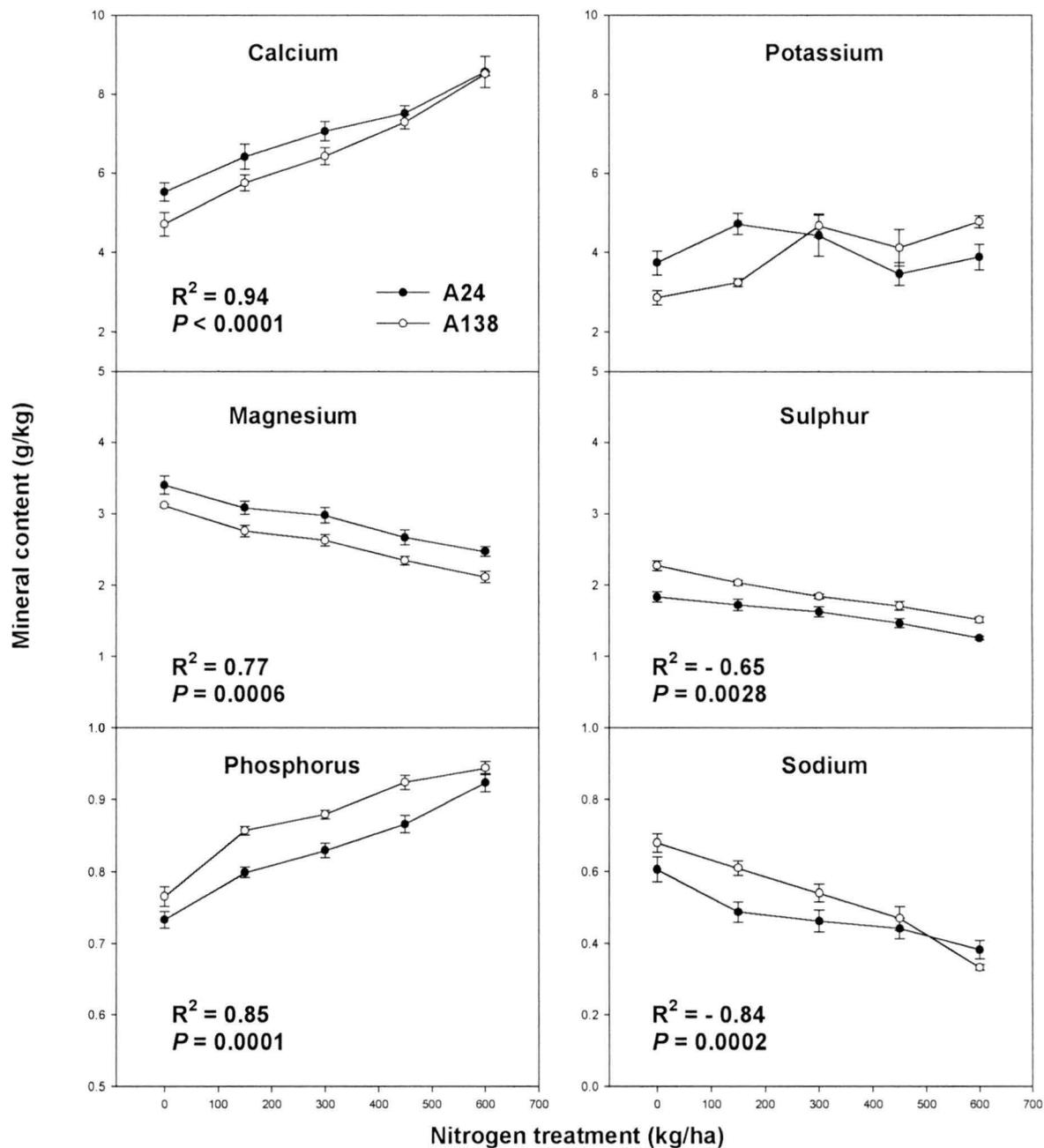
Fig. 3.5. Content of major elements of tested saltgrass accessions in response to different nitrogen treatments in the first season (2000). Bars represent the standard error. Note different levels of g/kg with element.

between Na content and nitrogen levels (R^2 = - 0.84). In general A24 had a higher content of Ca, P, K, and Mg while A138 had a higher content of only Na and S.

In the second season, Ca also was the predominant major element. It had a mean content of 8.13 g/kg in A24 and 8.49 g/kg in A138 which contrasts with its behavior in the first season where it was higher in A24. A linear relationship with nitrogen level was very clear (Fig. 3.6). The degree of association between the two variables was positive and highly significant (R^2 = 0.95). K was the second in dominance as in the first season with a slight difference between its mean content and S and Mg mean contents. K had a mean content of 2.34 g/kg in A24 and 2.95 g/kg in A138 with no significant difference between them. S mean content in A24 was 2.29 g/kg and 2.6 g/kg in A138 with a statistically significant difference. Mg had a mean content of 2.13 in A24 and 2.72 g/kg in A138 with a significant difference as well. There was no association between K content and nitrogen levels while there was a negative association between S, Mg and nitrogen levels (R^2 = - 0.82 and – 0.60 respectively). P and Na were present in relatively low amount in both accessions. P had a mean content of 0.84 g/kg in A24 and 0.95 g/kg in A138 with a significant difference while Na mean content was 0.41 g/kg in A24 and 0.45 in A138 which was also significantly different. P had a positive association with nitrogen levels (R^2 = 0.86) while Na had a negative association (R^2 = - 0.89). Generally in the second season all major elements were higher in A138 than in A24 contrasting with the behavior of Ca, P, K, and Mg in first season where their contents were higher in A24.
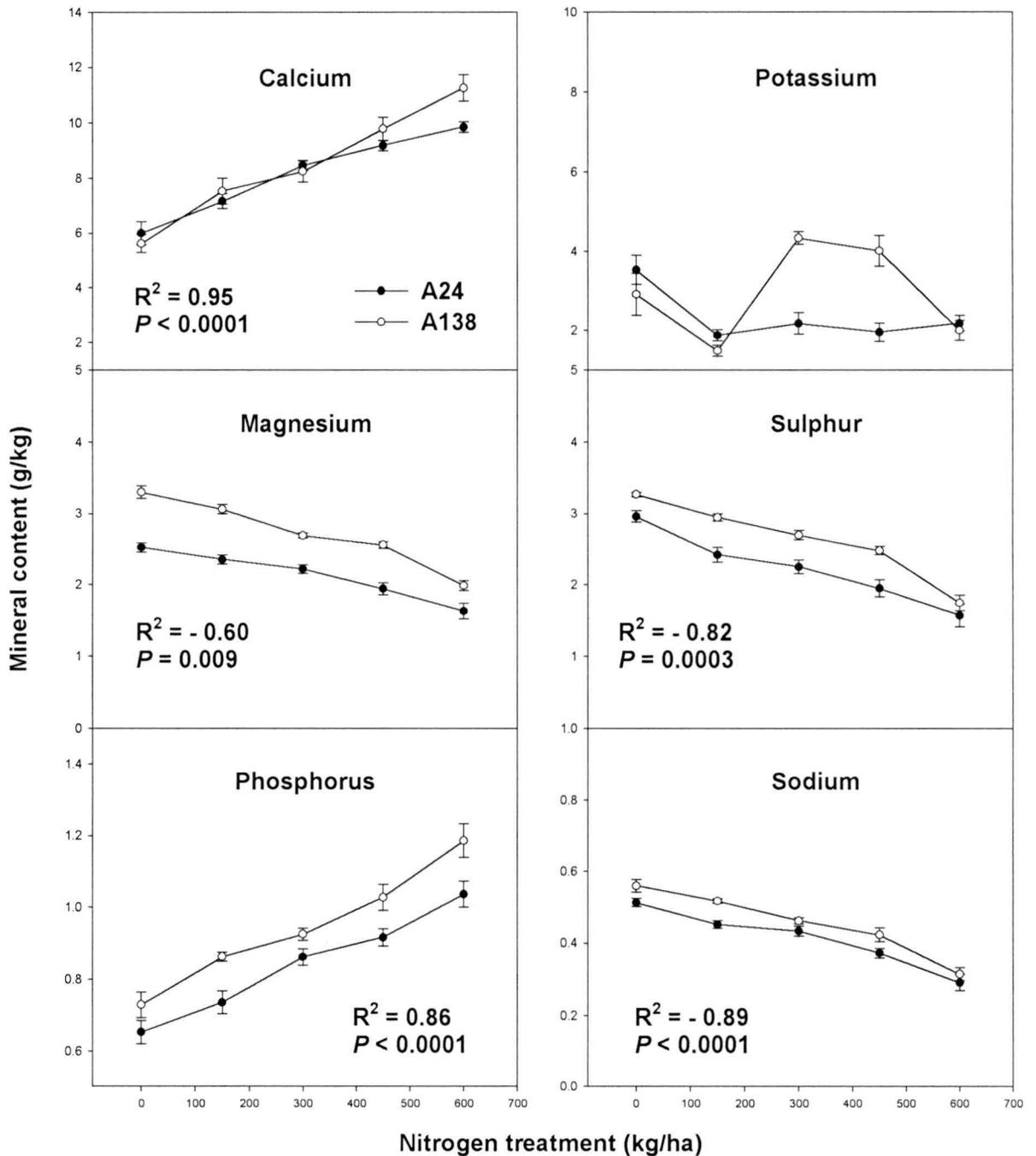
Fig. 3.6. Content of major elements of tested saltgrass accessions in response to different nitrogen treatments in the second season (2001). Bars represent the standard error. Note different levels of g/kg with element.

Minor elements:

McDonald et al. (1988) defined minor or trace elements as the elements present in the animal body in low concentration, i.e. not greater than 50 mg/kg. Minor elements quantified in this study were Fe, Zn, Cu, Mo, Mn, and Co. Analysis of variance indicated a significant difference in their contents between the two seasons, so each season is discussed separately.

In the first season, Fe and Mn had a relatively higher content than other minor elements (Fig. 3.7). There was no significant difference between accessions in Fe content which had a mean of 0.82 g/kg in A24 and 0.78 g/kg in A138 while a significant difference existed between the accessions in Mn content which had a mean of 0.25 g/kg in A24 and 0.17 g/kg in A138. A linear relationship between Fe and nitrogen levels was found (Fig. 3.7) with a significant positive association (R^2 = 0.91) while there was no association between Mn and nitrogen levels. The mean Zn content in A24 was 0.033 g/kg and was 0.034 g/kg in A138. Cu mean content in A24 was 0.012 g/kg and was 0.013 g/kg in A138. There was no significant difference between accessions in their contents. No association was found between Zn and Cu contents with nitrogen levels. Co and Mo contents were minute but sufficient in terms of animal body requirements. The mean Co content in A24 was 0.0005 g/kg and was 0.0004 g/kg in A138. Mo mean content in A24 was 0.0002 g/kg and was 0.0003 g/kg in A138. No association was found between Co and Mo contents and nitrogen levels. In this season A24 had higher mean contents of Fe and Mn and A138 was higher in Zn and Cu mean contents.
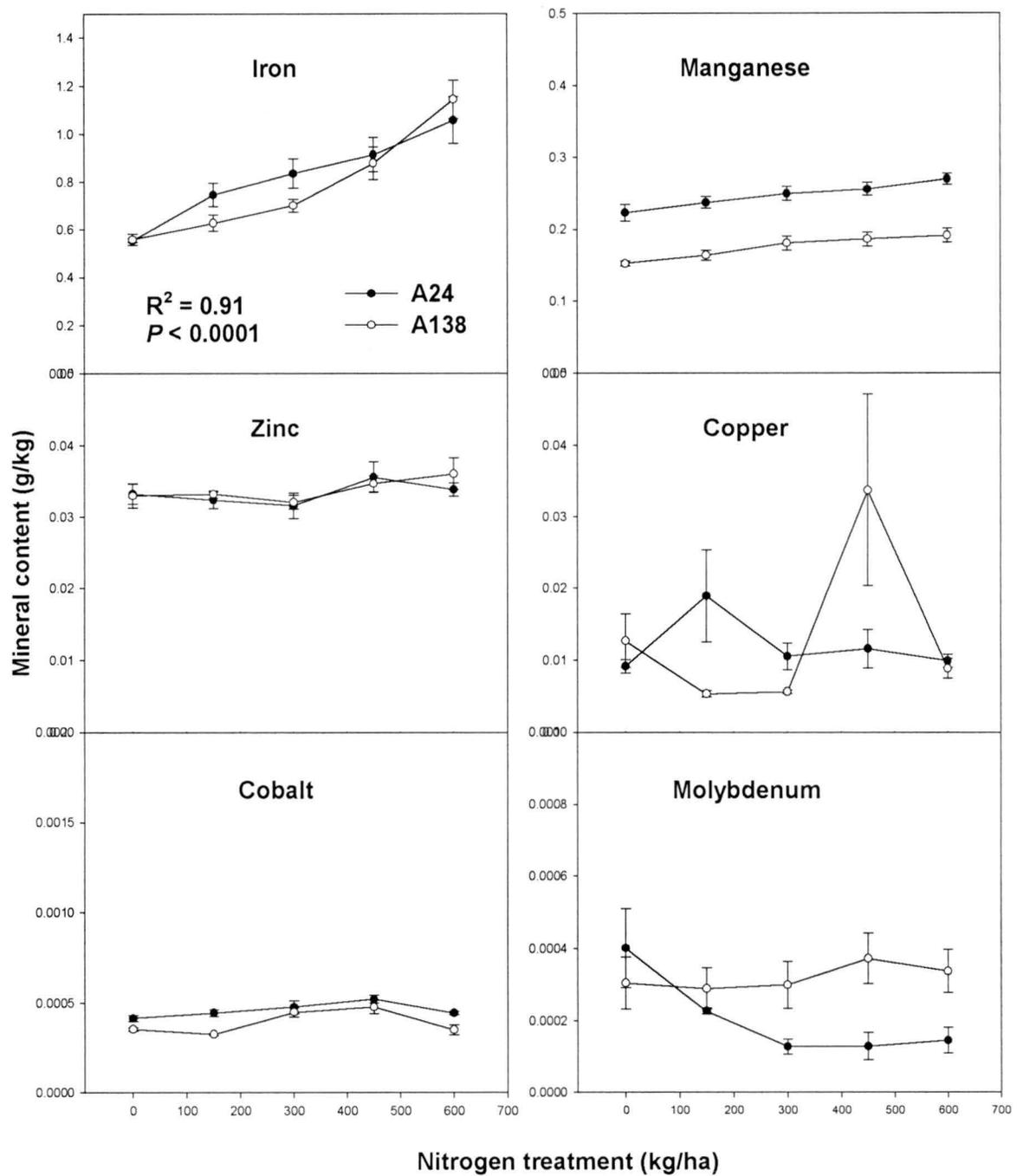
Fig. 3.7. Content of trace elements of tested saltgrass accessions in response to different nitrogen treatments in the first season (2000). Bars represent the standard error. Note different levels of g/kg with element.

In the second season, a similar trend as in first season was observed although there was a slight decrease in contents (Fig. 3.8). Fe had a mean content of 0.42 g/kg in A24 and 0.55 g/kg in A138 while Mn had mean contents of 0.096 and 0.18 g/kg in A24 and A138 respectively. Cu content in both accessions were half of those of the first season (0.005 and 0.006 g/kg in A24 and A138 respectively). Fe content showed a significant positive association with nitrogen levels ($R^2 = 0.78$) while Zn content showed a significant negative association with nitrogen levels ($R^2 = -0.64$). There was no association between Cu, Mo, Mn and Co contents and nitrogen levels. In contrast to the first season, A138 had a higher content of Fe and Mn and A24 had a higher content of Zn.
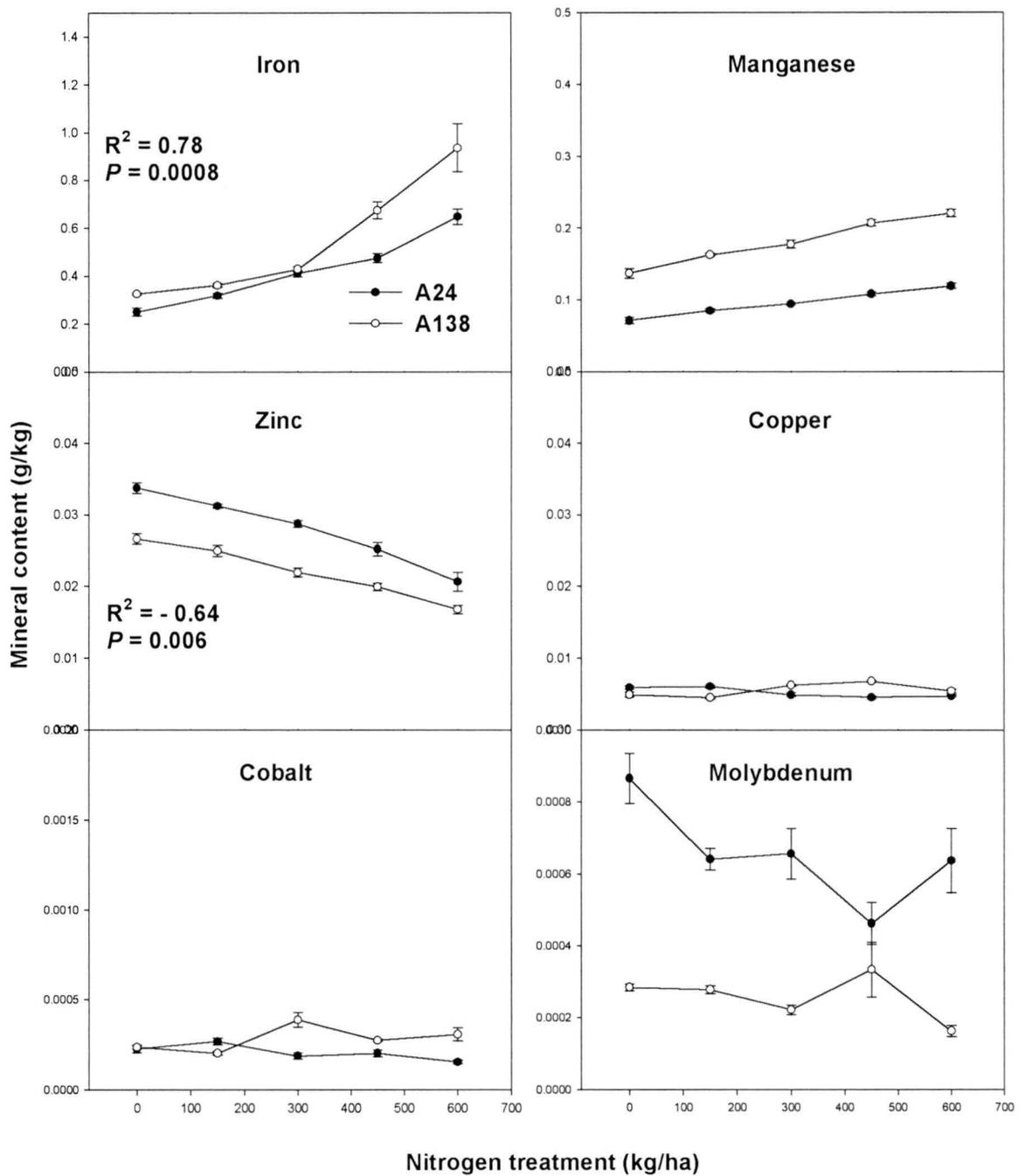
Fig. 3.8. Content of trace elements of tested saltgrass accessions in response to different nitrogen treatments in the second season (2001). Bars represent the standard error. Note different levels of g/kg with element.

DISCUSSION

Effect of nitrogen levels on saltgrass cover.

Soil water availability and nutrient gradients appear to affect plant establishment. During the first month of saltgrass establishment nitrogen levels had no effect. It seemed that water is the key factor during this month. Treatment effect was evident by the end of the second month. Treatment effect was positively linear until the end of the growing season where a threshold was approached at the rate of 450 kg/ha. These results stress on the importance of nitrogen fertilizer on saltgrass and agree with previous work on other grasses (Belanger and Gastal, 2000; Bittman and Kowalenko, 1998; Malhi et al., 1998; Munshaw et al., 2001; Oral and Acikgoz, 2001; Read and Hipp, 1998; Richard and Redente, 1995; Smart and Barko, 1980; Springer and Taliaferro, 2001). Both accessions responded well to nitrogen fertilizer but A138 showed a greater response although it was not statistically different.

Effect of nitrogen levels on saltgrass productivity.

We know of no other studies on the effect of nitrogen fertilizer on saltgrass productivity except the work by Smart and Barko (1980) who studied the effect of nitrogen nutrition on above ground biomass of *Distichlis spicata* (L.) and *Spartina alterniflora* (Loisel.). Although their results indicated a positive effect of nitrogen fertilizer on productivity, which agree with our findings, salinity interactions confounded their results as they investigated sediments of different salinity levels.

Nitrogen fertilization increased leaf area, and as a result, the conversion efficiency of intercepted radiation into shoot biomass (Belanger and Gastal, 2000). Studies on different

grasses showed a positive effect of nitrogen fertilization on productivity. Fluff grass (*Erioneuuron pulchellum*) exhibited a 51% increase at 25 kg/ha and a 100% increase at 100 kg/ha nitrogen amendments in a Chihuahuan desert (Ettershank et al., 1978). Productivity of *Stipa thurberiana* increased 49% in response to 45 kg/ha nitrate-N and 39% in response to 45 kg/ha ammonium-N (Miller et al., 1991). Hunt et al. (1988) suggested that primary production is N-limited in a shortgrass prairie, a mountain meadow, and a lodgepole pine forest. They reported 81%, 102% and a 52% production increase in the shortgrass prairie, the mountain meadow, and the lodgepole pine forest, respectively in response to 154 kg/ha N. The dry matter of tall fescue increased as well with increasing nitrogen rate on Houston blackland prairie soils at the Texas A&M University Research and Extension Center at Dallas (Read and Hipp, 1998). Other species such as chicory (*Cichorium intybus* L.) have shown an increase in dry matter production in response to nitrogen rate as well (Belesky et al., 2000).

The positive linear relationship between productivity and nitrogen levels indicated the importance of nitrogen for saltgrass productivity. The superiority of A24 over A138 in productivity may be related to morphological differences between the two accessions such as stolon diameter and density of growth or more upright growth since there was no significant difference in cover percentage observed.

Effect of nitrogen levels on nitrogen and protein contents.

The nitrogen content of bermudagrass clippings generally increased with nitrogen fertilization (Snyder and Cisar, 2000). Our results showed a positive linear relationship between nitrogen application rate and nitrogen and protein contents. Although A24 produced more dry matter than A138, the latter had higher tissue nitrogen and protein

content. This agrees with the general belief that forage nutritive value and dry matter yield are negatively related (Bregard et al., 2001). Results by Michaud et al. (1998) and Bregard et al. (2001) agreed with our results in that forage nitrogen content in timothy increased with nitrogen fertilizer.

Effect of nitrogen levels on mineral contents.

Many elements play essential functions in the animal body and thus must be present in the food. Some of them are bound to the enzymatic proteins, while others are present in prosthetic groups in chelated form. Elements such as Na, K, and Cl have an electrochemical function and play a role in the maintenance of acid-base balance and the osmotic control of water distribution within the animal body. Others have a structural role such as Ca and P, which are essential components of the skeleton. S is necessary for the synthesis of structural proteins. Mg plays more than one role. It functions catalytically, electrochemically and structurally. Fe is important as a constituent of haem, which is an essential part of some haemochromogens important in respiration. Co is a component of vitamin B_{12}. Some minerals are toxic in excessive quantities such as Cu, Se and Mo (McDonald et al., 1988). Supplementation of any diet with minerals should be done with care, especially with trace elements.

Ca was the predominant element in the two tested accessions. It is required in a relatively high amount in animal diet since its approximate concentration in the animal body is 15 g/kg. The range of 6.54 to 8.49 g/kg in saltgrass is high and make it a good source of Ca.

P was relatively low in both accessions in relation to its approximate concentration in animal body which is 10 g/kg. Therefore, we can not consider saltgrass as a significant source of P.

K, S, Mg and Na approximate concentrations in animal body are 2, 1-5, 0.4 and 1-6 g/kg respectively (McDonald et al., 1988). By comparing saltgrass accessions content of major elements with the ranges of contents of temperate pasture grasses given in Table 3.1, we can conclude that saltgrass accessions had a high content of Ca and Mg, a normal content of S, and low content of K and P. Na contents in A24 (0.5 g/kg) and A138 (0.6 g/kg) is higher than the mean contents of oats and barley, which is 0.2 g/kg and lower than the mean contents of lucerne, kale and clover hay which have a mean contents of 2.1, 2.0, and 1.9 respectively

Table 3.1. Essential mineral contents of two saltgrass accessions compared with the ranges of essential mineral contents of temperate pasture grasses.

Element	Range[*]			Saltgrass accession	
	Low	Normal	High	A24[**]	A138[**]
g/kg					
Potassium	< 12	15-30	> 35	3.7	4.6
Calcium	< 2.0	2.5-5.0	> 6.0	9.2	9.9
Phosphorus	< 2.0	2.0-3.5	> 4.0	0.97	1.1
Sulphur	< 2.0	2.0-3.5	> 4.0	2.4	2.8
Magnesium	< 1.0	1.2-2.0	> 2.5	3.0	3.2
mg/kg					
Iron	< 45	50-150	> 200	853	1038
Manganese	< 30	40-200	> 250	195	206
Zinc	< 10	15-50	> 75	34.7	30.7
Copper	< 3.0	4.0-8.0	> 10	7.9	7.8
Molybdenum	< 0.40	0.5-3.0	> 5.0	0.5	0.33
Cobalt	< 0.06	0.08-0.25	> 0.30	0.35	0.37

[*] Data presented after McDonald et al. (1988).

[**] Data presented is the average of 2000 and 2001 seasons.

(McDonald et al., 1988). Therefore, Na content in A24 and A138 are average when compared to other animal feeds.

Minor elements are required in very low concentrations for animal health. Approximate concentrations of Fe, Zn, Cu, Mo, Se, Mn, and Co in the animal body are 0.02-0.08, 0.01-0.05, 0.001-0.005, 0.001-0.004, 0.001-0.002, 0.0002-0.0005 and 0.00002-0.0001 g/kg respectively (McDonald et al., 1988). Depending on the ranges given in Table 3.1, saltgrass accessions had a high content of Fe and Co, a normal content of Mn, Zn and Cu and low Mo content.

In addition to the relatively reasonable essential minerals content, saltgrass had a relatively high protein content, which ranged from 7.8 to 11.8%. By comparing crude protein content in saltgrass accessions with the contents in other common animal feeds (Table 3.2), we found that saltgrass accessions had higher protein content than some green crops such as barley in the flower stage and maize, some silages such as whole barley and whole maize, and all grass hays either in poor or good quality, all straws and all cereals and their by-products except wheat bran. All these factors would indicate that saltgrass is a very promising range species and stress its importance in improving pastures in saline soils. This agrees with Cluff et al. (1983) who reported the importance of saltgrass as a forage species of the saline-alkali basins of the western United States.

Differences between seasons in productivity, nitrogen, protein, and mineral contents of the same accession could be related to physiological and metabolical activities, which are affected by environmental factors such as air temperature and soil water availability. Although irrigation was provided equally in both seasons, the higher precipitation of the first month of establishment, when water is critical, during the first season (June 2000)

could be related to the high productivity of this season (Fig. 3.9). Also, the relatively higher temperature of July and August of this season (Fig. 3.10) could have affected the performance of this warm season grass.

Table 3.2. Crude protein content of two saltgrass accessions compared with some common animal feed.

Food	Crude protein content (g/kg)
Saltgrass[**]	
A24	50-125
A138	70-144
Green crops[*]	
Barley in flower	68
Clover, red, early flowering	179
Clover, white, early flowering	237
Lucerne, early flowering	171
Maize	89
Rye grass, first cut	186
Timothy, early growth stage	184
Silages[*]	
Barley, whole crop	64
Lucerne	168
Maize, whole crop	110
Hays[*]	
Clover, red	184
Grass, poor quality	55
Grass, good quality	110
Lucerne, early flowering	225
Straws[*]	
Barley	38
Bean	52
Rye	37
Wheat	34
Cereals and by-products[*]	
Barley	108
Maize	98
Rye	124
Sorghum	108
Wheat	124
Wheat bran	170

[*]   **Data presented after McDonald et al. (1988).**
[**] **Data presented is the average of 2000 and 2001 seasons.**
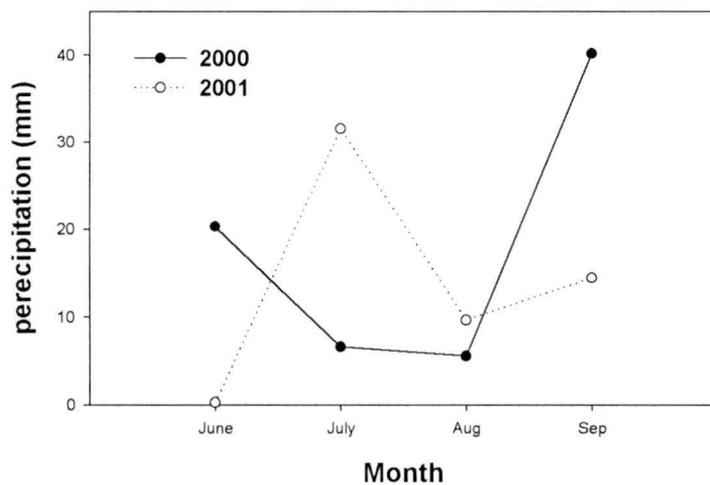
Fig. 3.9. Total precipitation during the growing seasons of 2000 and 2001.
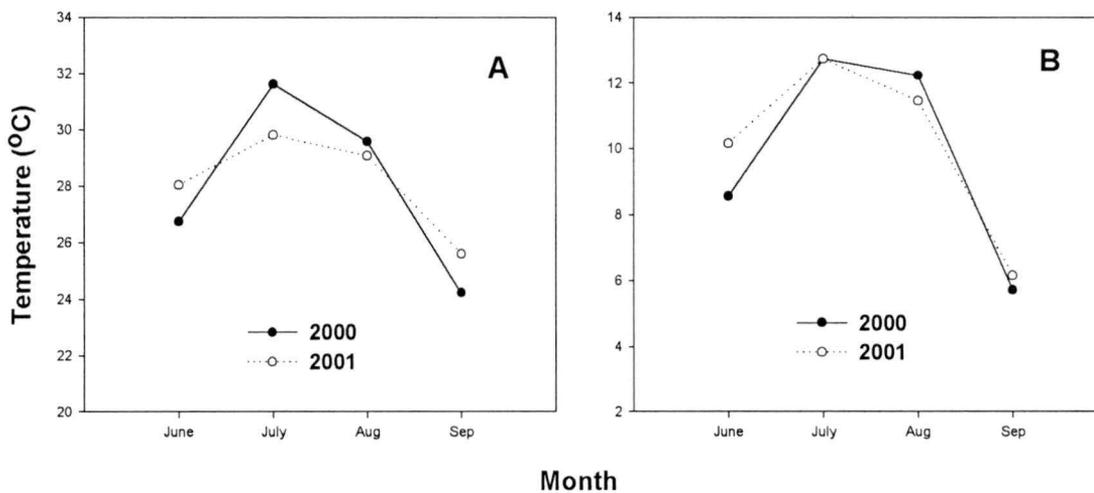
Fig. 3.10. Monthly average maximum (A) and minimum (B) temperatures during the growing seasons of 2000 and 2001.

Differences in mean contents of elements between accessions and alteration of their dominance from season to season indicated no interspecific differences between them. This could be related to the environmental conditions related to physiological and metabolical activities as nutrient uptake by plants differs according to the differences in transpiration rate and uptake rate of a given nutrient (Marschner, 1986).

In conclusion, nitrogen fertilization had a positive effect on establishment in terms of cover and productivity and nutritive value of the two tested saltgrass accessions. Nitrogen had no effect during the first month of establishment when water was critical. Nitrogen fertilizer level at 450 kg/ha showed the best cover percentage but the relatively small plug cover could influence the relative amount of N needed, i.e. N which falls on bare ground could be lost via leaching. Further, the high soil pH (8.3) of the study site may have resulted in higher nitrogen volatilization, especially with the use of granular urea as N fertilizer. Also, we recommend the use of saltgrass to improve pastures in saline areas and other areas where potable water is limited because of its high nutritive value.

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