EFFICACY OF SALINITY MITIGATION ON WARM SEASON TURFGRASSES

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by
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ABSTRACT

Salinity has become one of the major issues in turfgrass maintenance due to limitations on use of fresh water for turfgrass irrigation. Usage of brackish and reclaimed water cause damage to turfgrass and soil. Gypsum is widely used to mitigate the salt injury. The application of Gypsum on sand based root-zone is also noticed but the applicability of this was not initially designed for sand. The goal of this research was to evaluate the affect of additional gypsum on warm season turfgrasses under salinity stress. Poly-house and field experiment were designed in 2012-2014 and evaluation was carried out on ‘Zeon’ Zoysia grass, ‘Platinum’ Seashore paspalum and ‘TifEagle’ bermuda grass. Cultivars were established and salinity stress was imposed and application of gypsum was carried out to evaluate the differences.

Significant differences were observed for salinity and grass genotypes. turf quality, clipping yield, chlorophyll content decreased with increase in salinity and was not correlated to gypsum. The evapo-transpiration and electrolyte leakage were significantly reduced by gypsum application. Indicating that Calcium can be used as an nutrition but not as a sand amendment for salinity.

BIOGRAPHICAL SKETCH

Sathish Kumar was born in Tamil Nadu, south of India. He grew up in different states of the country, as his dad was in army and used to travel around a lot. During his travels he was fascinated by tea estates, coffee plantations, and golf courses. He was very interested in nature which made him to pursue his undergraduate in Horticulture at Tamil Nadu Agricultural University, Coimbatore, India. During his time in the university, Sathish was introduced to courses like landscaping and turfgrass management. To learn more about this Sathish conducted a study on identifying native grasses and problems faced by them.

Before graduating from TNAU, Sathish had an opportunity to apply for Cornell University, New York with support from Cornell - Sathguru foundation. He was accepted in August, 2012 and he began his work with Dr. Frank Rossi and Dr. Martin Petrovic in the Department of Horticulture. Sathish worked on salinity problems in turfgrass and its mitigation. In future, Sathish wishes to teach and research in the field of turfgrass and spread knowledge of turfgrass maintenance in India and around the world.

Dedicated to my dad, R. Sekar

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Chapter 1. Salt Stress and Mitigation Literature Review

ABSTRACT

Salinity has been one of the important problems in turfgrass industry for many reasons including the restriction to use fresh water for turfgrass irrigation. The restrictions and unavailability of fresh water has encouraged the turf managers to use brackish water, reclaimed or treated water for the turfgrass management. The other reasons include various interactions like soil properties, water quality, and location of the golf course.

Salinity is widely distributed among irrigated as well as non-irrigated regions of the world. Soil salinity and water salinity are the two main reasons behind salt stress in turfgrass. Soil salinity is due to cations like Na, Ca, K and anions like Cl, So_3, HCo_3. These cations breakdown soil and deflocculates soil clay particles resulting in compaction and drainage problems resulting in physiological problems in the turfgrass site. Salts in water influence the Electrical Conductivity (EC) of the water, which causes salt stress by stripping of cations from the cell wall of the turf. This problem is more common in sites along the ocean as they frequently incur salt spray from the ocean.

In recent years, the golf putting greens and athletic fields are constructed based on United States Golf Association (USGA) sand based root-zone mix and California root-zone mix and these mixes has very few clay content or organic matter in them. At any particular time the sand has limited Cation Exchange Capacity (CEC). Any addition of ion to them will only replace the already present ion in the exchange sites.

Salinity causes physiological drought due to compaction of the root-zone. It also causes thinning of turf cover thereby reducing the turf quality. Reduction in shoot growth is noticed and up to moderate salinity the root growth increases and then declines at higher end of salt stress leading to death of turfgrass.

The mitigation technique like application of gypsum is followed widely in the turfgrass industry for salinity stress. The Ca in the gypsum replaces the Na in the soil and results in flocculation of clay particles thereby improving the infiltration of water in the root-zone. Even though this works on soil based root-zones the applicability of this technique on sand based turf is unknown.

The goal of this research is to elucidate the physiological responses such as electrolyte leakage, ion accumulation to salt and gypsum application. Specifically, this project focuses on the turfgrass visual quality, performance under salt stress, and after mitigation through gypsum application.

INTRODUCTION AND LITERATURE REVIEW

Urban development has increased the demand for fresh water supply and this has prompted state as well as local government on restricting the use of fresh water for turfgrass irrigation. The availability of potable water is decreasing with the growth of population (Marcum, 1994). A 18-hole golf course uses about 250,000 and 1,000,000 gallons of irrigation water per day for maintaining the turf (Huck et al., 2000). Using waste water for irrigation helps the turf managers to reduce the operating cost (Cuthbert and Hajnosz, 1999). As turfgrass sites are considered as suitable places for use of alternative water source of irrigation. It resulted in use of brackish water, ocean water, and treated water for the turf grass management (Dudeck et al., 1983).

In North America alone, 68,500 sq mi of area are affected by salt (Carrow and Duncan, 1998). Turf managers face various issues due to use of saline water like soil salinization, salt injury to turf, loss of soil structure due to sodium, and bicarbonate effects (Marcum, 2006). Salinity may arise because of one or various interactions which includes water quality, climate, soil properties and irrigation water during evapotranspiration (Al-Harbi et al., 1992).

Salinity is one of the widely distributed abiotic stress in irrigated as well as non-irrigated areas of the world (Ashraf et al., 2008). Thus, the salinity can be classified as (a) soil salinity and (b) water salinity. Soil salinity is due to cations like Na^+, Ca^{2+}, K^+, and anions like Cl^-, So_4^{-2}, CO_3^{-2}, HCO_3^-. High sodium content in comparison to calcium in soil deflocculates the clay particles which results in poor drainage and reduced soil structure (Marcum, 1994). Salts in soil solution can induce physiological drought and hinder or reduce the water uptake by turfgrass. The salts in water influence the Electrical Conductivity (EC) of the irrigation water. Irrigation water containing soluble salts of about 480 parts per million or 0.75 deciSiemens/meter (dS/m) can cause salt stress to the turf (Carrow and Duncan, 2004). The golf courses established near the coastlines are exposed to salt water with high EC. Thus, the damage due to salt spray in turfgrass has increased (Carrow and Duncan, 1998). Resulting in the development of salt tolerant turfgrass in recent years, specifically halophytic grasses allowing the use of saline water or reclaimed water in a broad way (Duncan et al., 2009).

Salinity in Turfgrass

Golf course greens and athletic fields are constructed based on United States Golf association (USGA), using sandy rootzone mix (Snow, 1993). Christians (1990) found that these sands may be calcareous or silica based having Cation Exchange Capacity (CEC) of 1 to 6 cmol/kg. Although calcareous sand contains 10 to 40% free $CaCo_3$ by weight it is far greater than what the silica based sand.

United States Salinity Laboratory (USSL), classifies salt affected sites as: saline, sodic, and, saline-sodic. Soil with high soluble salts are categorized under saline soil. The sodic soil has high exchangeable sodium (Na) and saline-sodic soil has both soluble salts as well as exchangeable sodium at greater quantity.

Carrow and Duncan (1998) reported white alkali soils or saline soils with EC of 4 dS m-^{1} and Sodium Absorption Ratio (SAR) less than 12. SAR is the measure of sodium ions in relation to calcium and magnesium ions. It represents the sodium status of the soil as it is most harmful to the plants and soil. Sodic soils has high quantity of sodium resulting in deflocculation of clay particles in the soil. These soil have SAR ≥ 12 and EC less than 4 dS m-^{1}. The saline-sodic soil has EC greater than 4 dS m^{-1} and SAR ≥ 12. They affect the plants by creating osmotic stress and reducing the water uptake by plants through roots (Carrow and Duncan, 1998).

The major salt contributing to soil salinity is Sodium chloride (NaCl) (Jungklang et al., 2003). Salt affected sites has greater exchangeable Na, high level of soluble salts, or both. Rubinigg et al. (2003) reported that high NaCl, in saline soil and irrigated water suppressed the uptake of essential cations and anions. High salt content is also responsible for ion toxicities and ion imbalance leading to nutritional problems.

St. John and Christians (2010) demonstrated the difficulties in obtaining ‘ideal-ratio’ of cations in sand-based media with low-CEC. The Basic Cation Saturation Ratio (BCSR) theory which states that “ there is an ‘ideal-ratio’ of the basic cations Ca, Mg, and K, and when the ratio is not ideal, fertilizer application must be made to promote the plant health”. Graham (1959) further included a range of ideal equivalent percentages, Ca 65-85%, Mg 6-12%, and K 2-5%. According to Nelson (1991) any excess addition of cation to a sand-based media may shift the concentration of other cations in the sand. This is known as nutrient antagonism. Thus, at any particular time there will be limited number of cations that each sand can hold. Hence the percentage of each ions on the exchange complex will change frequently (St. John et al., 2001).

Addition of a cation and its affect on other cation is noticed only when the cation ratio is small. As St. John et al. (2001) reported that the affect of Ca fertilization on Mg was not noticed on silica sand compared to calcareous sand, as silica sand had larger Mg to Ca ratio. Spencer (1954) said that the K mobility in sands is high compared to Ca and Mg. They often tend to leach out through the root-zone (Lodge and Lawson, 1993) resulting in multiple K application (Carrow et al., 2001). Literature shows contradicting results for cation application and turfgrass performance in both calcareous and silica sand (Turner and Hummel, 1992).

Salinity Affects on Turfgrass

The ions causing toxicity problems are Na, Cl, and B. Irrigation water with high Na strips Ca from the shoots. The shoot cell wall are negative in charge resulting in CEC sites having Ca. The Na in water displaces the Ca in the plasma membrane and the turgor pressure is lost. It results in leakage of K from the cell and the osmotic adjustment potential is lost. The increased salinity exposure leads to calcium deficiency, exhibiting yellowing of the older leaves in turfgrass (Carrow and Duncan, 2011).

Routine mowing removes the accumulated ions in the turf canopy. Thereby, reducing the risk of salt injury. Root cells bind Ca to CEC sites of the root cell wall. It results in competition between Ca and Na for the cell wall exchange site causing root damage through cell wall deterioration around root tips (Carrow and Duncan; Best Management Practices for Saline and Sodic Turfgrass Soils: Assessment and reclamation, CRC press, USA 2011).

Much research is conducted on the physiological response of turfgrass to increased salinity and literature is available. The thinning of turf canopy is often noticed under salinity stress. Some halophytic grasses show increased root growth under low salinity stress (Marcum and Murdoch, 1994). Harivandi (2004) states that irrigation water with high levels of sodium causes damage to leaves when absorbed by leaves resulting in fertilizer burn. Wu et al. (1999) also found that overhead irrigation with salt water resulted in significant damage to the turf when compared to hydroponic system with same salt concentration.

Koch and Bonos (2011) evaluated the salinity tolerance screening methods: an overhead irrigation greenhouse method, hydroponics system, and field screening method for cool-season turfgrasses. The field screening method had lower percent green value compared to hydroponic system. Significant correlation was found between the two greenhouse methods for dry clipping, root, and shoot weight. Out of the three screening methods the field method had lower reliability compared to other methods due to the unpredictable conditions. Nevertheless, it is beneficial as it represents the true challenge faced by the turfgrass in real growing environment.

Plant Response to Salinity

Plants have different mechanism to tolerate salinity. These include increased root growth, ion exclusion, osmotic adjustment, compartmentalization, glandular secretion and formation of compatible osmolytes (Marcum, 2008a). Removing salt from shoot tissue through different means is associated with overall salinity tolerance (Qian et al., 2001). Thus, the ability to exclude Na and Cl has been used to classify the cultivars based on salinity tolerance. The excretion glands are found in the abaxial as well as adaxial surfaces of the leaves (Marcum, 1999). Salt gland density and excretion rates are found to have direct correlation with the salinity tolerance of the turf grass cultivars (Marcum and Murdoch, 1994). Marcum and Pessarkli (2006) found that the ion secretion was correlated with intra specific salinity tolerance of Zoysia and Bermuda grass cultivars.

Salinity stress results in physiological drought and to overcome this the turfgrass produces osmolytes for osmotic adjustment, thereby regulating the osmolarity of cell cytoplasm to prevent the loss of water (Hellebust, 1976). The imbalance created in plant due to high Na+ ion concentration is overcome by maintaining a high K+/Na+ ratio (Marcum and Murdoch, 1990a). Examples of compatible solutes in plants include glycinebetaine, proline, trigonelline, polyols, and cyclitols (Gorham, 1996).

Flowers (1985) found that turfgrass accumulate the harmful ions in vacuoles which accounts for more than 90% of the plant cell. This helps in preventing the potential damage that the ions would cause to the proper functioning of the plant. Studies have found that root growth is stimulated under moderate salinity stress. The roots are responsible for water uptake and the water transpired by the root tissue. Thus, an increase in root/shoot ratio takes place with respect to the osmotic stress caused by the high salinity (Dudeck et al., 1983; Gorham et al., 1985). The grass species such as bermuda grass (Cynodon spp.) (Dudeck et al., 1983), seashore paspalum (Paspalum vaginatum Sw.) (Dudeck and Peacock, 1985a), Manila (Zoysia matrella (L.) Merr.) (Marcum and Murdoch, 1990a) have shown significantly higher root growth compared to the control plants under salinity stress. But in high salt conditions, reduction in root growth have been observed.

Salinity response of Bermuda Grass **(*Cynodon spp.* Rich*.)***

Bermuda grass widely used warm season turfgrass in the world. They are sterile triploid, fine textured grasses used for golf courses. Tifdwarf and Tifgreen were most salt tolerant and shoot growth reduced by 22 % and root growth increased by 270% at the highest salt level. Regression analysis within cultivars increased Na and decreased K while total Na plus K in top growth was un-affecetd by salt concentration (Dudeck et al., 1983). Ackerson and young (1975) found 50% top growth reduction relative to control in cv. Santa Ana when exposed to 160 meqL^{-1} of a 50/50 mix of NaCl and $Cacl_2$ for 6 weeks. Marcum and Murdoch (1994) reported that the bermuda grass cv. Tifway (*Cynodon dactylon x C. transvaalensis* Burtt-Davey) showed intermediate tolerance to salinity with reduction in 50% shoot growth at 270mM salinity. The salinity tolerance was achieved by excluding the Na^+ and

Cl^- ions from the shoots with the aid of salt glands, a common trait in the grasses of the subfamily Chloridoideae (manila grass, bermuda grass, and Japanese lawngrass).

A field experiment by Pasternak et al., (1993) showed that the bermuda grass cv. Suwannee is more salt tolerant than seashore paspalum at Ec_{iw} of $14dSm^{-1}$. Also two bermuda grass selections from Oahu, Hawai, were more tolerant than Tifgreen (Marcum and Murdoch, 1990b). In an another study by Loch et al. (2010) the salinity tolerance of *C. dactylon* cultivars overlapped with that of *S. secundatum* group. Dudeck et al. (1983) studied the effect of sodium chloride on *Cynodon* turfgrasses and found that the top growth decreased with increasing salinity but the root growth increased by 270% at highest salt level of 9.9 dSm^{-1}. The cultivars differed in their response when salinity was increased up to 32.5 dSm^{-1} , 'Tifgreen' and 'Tifdwarf' were most tolerant compared to 'Common' and 'Ormond' which were most sensitive. 'Tifeagle' had lower turf quality (6.1) compared to 'Champion' (6.9) at 12.90 dSm-1 after 10 weeks of application (Bauerle and Toler, 2006). *Cynodon dactylon* (satiri) suffered a 50% shoot and root growth reduction at 30.9 and 33.4 respectively (Uddin et al., 2012).

Al-Khalifah (2004) studied the response of bermudagrass cultivars 'Tifway' and 'Tifgreen' at two salinity levels (4.6 and 10.72 dSm^{-1}). 'Tifway' produced higher amount of biomass at high salinity and appeared to tolerate the increased salt concentration in the soil. Bermuda grass showed good turf quality with respect to color when compared with Zoysia and St. Augustine under similar salinity conditions in a greenhouse. Pessarakli et al. (2008) found that the canopy color changed to lighter green for bermudagrass as salinity stress increased from 7000 to 21000 mg/L NaCl. Marcum and Pessarakli (2006) found that salinity tolerance in bermudagrass turf cultivars were based on salt glands excretion rate, which are present in both abaxial and adaxial surfaces of all the cultivars. The 50% shoot weight reduction of different cultivars ranged from 26-40 dS m^{-1}. Thus, Bermuda has wide range of cultivars tolerating various salt regimes hence it is wise to evaluate them up to sea water level, Ec_w = 54 dS m^{-1} or 34,560 mg/L (Duncan and Carrow, 1999).

Salinity response of Zoysia Grass **(*Zoysia spp.*)**

Zoysia grass consists of several species which are separated based on texture and cold tolerance: Japanese lawn grass (Zoysia japonica Steud.), Manila grass (Zoysia matrella [L.] Merr.), and Mascarene grass (Zoysia tenuifolia Willd. ex Trin.) that are being used as turfgrass (Murray and Engelke, 1983). Harivandi et al. (1992) classified zoysia grass as salt tolerant compared to other turfgrasses tolerating up to 16 dSm^{-1}. Emerald hybrid zoysia grass is found to me more salt tolerant than most of the warm season turfgrasses (Dudeck and Peacock, 1985). Salinity tolerance is measured using the 50% growth reduction in shoots. Uddin et al. (2011) conducted salt tolerance studies on various turfgrass and the result indicated that Zoysia japonica suffered from 50% shoot growth reduction at 36 dSm^{-1} and root growth reduction at 44.9 dSm^{-1}, which were higher than St. Augustine, bermudagrass, bahiagrass, pearl blue, and serangoon grass.

In decreasing order of salinity tolerance Emerald> FSP-3 seashore paspalum > Tifway bermudagrass>FSP-1 seashore paspalum>bermuda grass cv. Tifway II> Floralawn St. Augustine grass>Common centipede grass>Argentina bahia grass (Dudeck and Peacock, 1985). Marcum and Murdoch (1994) found that 50% reduction in shoot dry weight was higher for Japanese lawn grass compared to Manilla grass grown in solution culture up to 12 dSm^{-1}. Sharon et al. (1998) reported that the percent relative leaf firing varies between 19-80% at 400mM NaCl for the various species of Zoysia grass. Thus, indicating a wide range of salinity tolerance. The salinity tolerance between two zoysia grass species was related to shoot Na^+ and Cl^- exclusion, due to variation in salt secretion from leaf salt glands (Marcum and Murdoch, 1990b).

Marcum et al. (1998) found that Japanese lawn grass cv. Meyer to be equivalent to bermuda grass cv. Arizona Common in salinity tolerance. Z. *koreana* was found to be most salt tolerant, followed by Z. *sinica*, Z. *matrella*, and Z. *japonica* . Among Fifty-nine zoysia grass species evaluated for salt tolerance in solution culture by Marcum et al. (1998), Diamond Manila grass was found to be most tolerant and superior to El Toro, Belair, Meyer, Emerald zoysia hybrid grass, and Korean common Japanese lawn grass.

Salinity response of Seashore paspalum **(*Paspalum vaginatum* Swartz*)***

It is a perennial warm season turfgrass, and native of tropical and sub-tropical regions (Turgeon, 2011). Among the C4 grasses used as turf, seashore paspalum is the most salt tolerant up to 20 dS m-1 (Carrow et al., 2001). Henry et al. (1979) found paspalum cultivars to survive in soils with Ec_e of 45dS m-1. A variation in salinity tolerance of seashore paspalum accessions were noted by Pasternak et al. (1993), where bermuda grass cv. Suwannee performed better than paspalum. Similarly, Dudeck and Peacock (1985a) found Emerald Zoysiagrass hybrid to be more salt tolerant in comparison to FSP-1 and FSP-3. FSP-1 was most salt tolerant, with 50% shoot growth reduction at EC 28.6 dS m-1, followed by Futurf and FSP-2, and Adalayd.

Shahba et al. (2012) studied salinity affects on salam, Excalibur, and Adalayd cultivars at different mowing heights and reported that Salam had higher clipping yield and greater photosynthetic rate(Pn) compared to other two cultivars. The total nonstructural carbohydrate (TNC) content decreased while reducing sugar content (RSC) and proline increased with salinity. At highest mowing level, the root mass increased from 150 to 200 % for Saalam, Excalibur and Adalayd with increase in salinity from 0 to 44 dSm-1. As Salinity level increased the proline level increased up to 400%. Paspalum had highest K/Na selectivity at 45mm height. Increased mowing height increased salinity tolerance.

Irrigating ‘SeaDwarf’ seashore paspalum with non potable water of salinity ranging from 0.52 to 49.40 dSm-1 showed that the turf quality was better for pots irrigated with lower levels of salinity (Berndt, 2007). Shoot and root lengths and shoot dry matter weights decreased slowly with increased salinity. The canopy color changed to lighter green as salinity increased from 0-21000mg/L NaCl (Pessarakli et al., 2008).

The cultivars Aloha and SeaDwarf of Seashore paspalum under hydroponic condition did not show any symptom of stress up to 15,000 mg/L NaCl salinity level but with increasing salinity the shoot and root growth reduced. Also the visual quality deterred and dry matter production was less but they substantially reduced the salinity level of the culture rhizosphere (Pessarakli and McMillan, 2014). The turf quality, relative water content, and leaf photochemical efficiency decreased and electrolyte leakage increased when the paspalum grass was exposed to salt regimes of 300 and 500 mM (Liu et al., 2011). Hawaii selection showed 50% shoot growth reduction at 400 mM salinity (Marcum and Murdoch, 1994). This indicates a wide range of salinity tolerance in seashore paspalum cultivars.

Nutrient Use On Salt Affected Site

Carrow et al. (1998) in his book Salt-affected turfgrass sites: assessment and management explains that excessive Na reduces soil permeability and effects the growth of turf grass, the Na in the soil or irrigation water will require that chemical amendments to be used to add Ca as a replacement ion to the soil. Any salt effected soil with high Na will need amendment treatment to provide Ca to the soil. The most common chemicals added to increase the ratio of Ca to Na are gypsum, elemental S, or sulphuric acid.

Gypsum is widely used in sodic and saline-sodic amendment to improve the plant growth by reducing the sodium concentration and the exchangeable sodium percentage (ESP) of the soil, and by improving soil water movement and aeration (Carrow et al.,2001). It is found in the form of dehydrate, hemihydrate, anhydrite, phospho-gypsum or flue gas desulfurization gypsum. The solubility of gypsum depends upon the crystal size. Addition of gypsum ($CaSO_4$) allows the Ca^{++} to release and replace the soil-bound Na^+. The released Na^+ is leached out as Na_2SO_4, and the soil tends to granulate due to flocculation (fluffing up and colloidally glued together on the microscopic level) with more Ca^{++} on the exchange sites. This granulated condition improves soil structure, and soil is then less prone to compaction.

Frequently, application of gypsum is specified for turfgrass grown in native sand or sand based greens, even though such technology was not developed for use in sand soils. When soil test results show high sodium levels it increases the concern regarding the fact that that the soil is not sodic (15% or more of the cation exchange capacity occupied by Na), or saline (conductivity of saturation extract>4dS m-1). When treated with gypsum on sand based turf grass it did not affect pH, or extractable Ca, P or K. It in turn reduced the Mg content in the soil. The gypsum application did not affect the SAR or extractable Na. When continuously applied Gypsum produced minor changes in the soil total pore space, Micropore space and in water holding capacity. George et al. (2004) stated that repeated application of gypsum at sand based burmudagrass did not affect the growth or soil physical properties, even though appreciable Na was supplied in the irrigation water. For these Non sodic sand soils, Na did not adversely affect the grass performance, and the use of gypsum appeared to be unwarranted.

Sand based atheletic fields and golf course greens may contain large amount of Calcium carbonate. Calcium is frequently applied on those fields when irrigated with salt water. Treatments include application of different forms of calcium. Numerous studies conducted on sand based turfgrass demonstrated that additional Ca application were not needed (St.John et al., 2001). Even crops grown on quartz sand culture has shown that Ca fertilization can increase leaf Ca content without improving the health, growth and color(Spiers,1993; Spiers and Braswell,1994). Therefore we can conclude that sand has very small CEC, limiting the number of cations it can hold at any one time. Hence the percentage of each ion on the exchange complex can change. Adding an excess cation to any sand based medium will shift the concentration of other cations. This competition for exchange of sites cause the Mg deficiency in the leaf. The reduced saturation percentages and soil-exchangeable concentrations of Mg and K could eventually cause plant deficiencies. Many studies have concluded that maintaing the minimum critical level Ca and Mg is more important (Saratin,1985). Hence the study regarding the use of nutrients in sand based turf grass is essential. As the media doesn’t have any significant clay but during the growth of the plants and in the rootzone the accumulation of organic content increase and the exact value of nutrient to be applied is not determined. Further studies are required to study the effects of regular nutrient application in the sand based turf grass and to estimate the new or technical assistance required to tackle the high salt content in the sand based turf.

Thesis Objectives

Nutrient (Salt mitigation) use on sand based putting greens and athletic fields irrigated with saline or brackish water has been followed for some time; yet a clear understanding of how the gypsum affects the turfgrass performance, quality and root zone is lacking. There is need to study the performance to justify the widespread adoption of this method of salt mitigation in the turfgrass industry. The fundamental studies were performed in growth chamber and greenhouse conditions to evaluate the salinity stress affects on turfgrass physiological response to mitigation practice.

The second core research goal was to understand how the calcium application effects whole plant physiology under open field experiment. Specifically, common turfgrass quality parameters were evaluated. Turfgrass electrolyte leakage, TNC, proline, phenol, and cation concentration in shoot as well as soil were evaluated to develop best management practices for turfgrass under salinity stress.

Chapter Two: Calcium effects on water consumption, clipping yield and quality of turfgrass under different salinity levels

ABSTRACT

Turfgrass performance under salt stress is studied world wide and mitigation techniques are suggested to alleviate compaction and other problems caused by salt. Calcium application is one of those mitigation techniques that is widely being used in the turf industry. Field observations and trade reports suggest that calcium or gypsum is used on pure sand based root zone, for which this mitigation system was not designed. The effect of calcium on turfgrass grown in sand, irrigated with sodium rich water have not been evaluated scientifically. Pot studies were conducted on 'TifEagle' bermudagrass, 'Zeon' zoysiagrass , 'Platinum' seashore paspalum under salt stress condition. Salt concentrations of 0.0, 10.0 and 20.0 dS m^{-1} was used and calcium (gypsum) application of 0.0, 11.0 and 22.0 g Ca m^{-2} was done at biweekly intervals, to asses the affect of calcium on clipping yield, turf quality and water consumption of turfgrasses under salinity stress. This study was a completely randomized design with factorial arrangements, each treatment was replicated four times. Data on clipping yield, water consumption, and turf quality was measured and means were compared using LSD test at 5% level. Regression analysis indicated that salinity had significant affect on turf quality (TQ), Platinum and Zeon had greater TQ (7.29 and 7.19) compared to TifEagle (6.84).

For increasing salinity level from 0 to 20 dS m^{-1} the clipping weight for genotypes decreased in the order of Zeon> Platinum> TifEagle. The values of evapotranspiration (ET) decreased from 0.78 mm, 0.62 mm and 0.39 mm for Platinum> TifEagle> Zeon for gypsum application of 0 to 22 g Ca m^{-2} on grasses. ET decreased significantly with increase in gypsum application @ 11.0 g Ca m-^{2} and 22 g Ca m^{-2} by 0.61 mm and 0.53 mm.

INTRODUCTION

Clipping yield is an important measurement to evaluate the performance or growth of grass and can be influenced by mowing practices, grass selection, fertilization, and water management (Marcum and Murdoch, 1990a). Some grasses, due to their innate ability are able to tolerate some level of stress beyond which the reduction in clipping yield takes place (Carrow and Duncan, 1998). The major stress faced by turfgrasses around the world include salt stress and drought stress. Turfgrasses can tolerate up to some level of stress and that is called as threshold of the turfgrass. Turfgrasses show reduction in clipping yield and to mitigate the loss calcium is applied to grasses. The effect of calcium on clipping yield is not studied thoroughly and very little literature is available. Although calcium addition to Kentucky blue grass on calcareous sand did not increase clipping yield, it increased clipping yield by 15% for creeping bentgrass (John et al., 2001). When gypsum applied to creeping bentgrass grown on silica sand, the clipping yield increased by 32-52 % (John et al., 2001). On an average the grasses grown on silica sand produced more dry clippings when compared to grasses grown on calcareous sand with additional calcium treatments. Under salt stress the clipping yield decreased with increasing salinity (Shahba et al., 2012). The decrease in plant biomass is due to low water potential, specific ion toxicity (Greenway and Munns, 1980). Turf quality is a major component of aesthetic quality. Traditionally turf quality have been evaluated based on visual ratings , on a scale of 1 to 9. Yellow to brown representing 1 and 9 representing good turf (Karcher and Richardson, 2003). Literature suggests that turf quality decrease is inversely proportional to salinity for most of the turfgrasses and quality plays an important role to asses the performance of grass under stress. A study by John *et al.* (2001) reported that additional Ca to grasses grown on silica sand is beneficial. The effect of calcium on turf quality with salinity problem were not studied briefly.

The goal of this project was to evaluate the calcium effect on clipping yield, water consumption, and its influence on turf quality. The first objective was to determine if calcium improves the turf quality and clipping yield of the turfgrasses. The additional objective were to determine how salinity and calcium affects water consumption by the grass. Finally interactive effects of calcium application and salt applied on turfgrasses were evaluated.

MATERIALS AND METHODS

Experiment Description

This study was conducted in Guterman green house, growth chamber facility and poly-house facility at the Cornell University, Bluegrass Lane Turf and Landscape Research center in Ithaca, NY initiated in 2012. Three different warm season salt tolerant turf grass ‘TifEagle’ Bermudagrass, ‘Zeon’ Zoysiagrass and ‘Platinum’ Seashore paspalum grass were selected for their excellent turf quality, salt tolerance, and difference in salt tolerance mechanism. They were vegetatively propagated from plugs. After removing soil from roots by hand washing using ‘Calgon’, the grass plugs were transplanted to cylinder type lysimeter pots (46 x 10 cm) constructed from polyvinyl chloride pipe (PVC), fitted with base. A small opening was made near the base for drainage purpose. Pea gravel (3 to 6 mm diameter) were put up to 0.5 cm at the bottom of the pots for drainage purpose. To ensure that the pots are uniformly packed with sand, the process was divided into two parts. First the pots were fully filled with the sand and was wetted than allowed to settle down. After that the next portion of sand was added and the pots were saturated and allowed to drain for 24 hours before planting. The sand used was ‘disney sand’ as it is an inert material and doesn’t have any salt to interfere with the study.

The pots were initially grown in Gutermann green house growth chamber facility under 80ºF/70ºF day/night temperature and 400 ppm N (3 times a week). Full density canopy was achieved in 8 weeks for ‘Platinum’ and ‘TifEagle’ but the ‘Zeon’ had to be grown under 85ºF/80ºF day/night temperatures and 600 ppm N to achieve good density. The grass were clipped 4-5 times a week and watered twice a day with regular tap water. Occurrence of sucking pests were taken care by yellow sticky trap. Once the grasses were fully grown they were transferred to ‘Bluegrass Lane Turf and Landscape Research Center’, Cornell University.

Experimental Design

The experimental design is a randomized complete block design with 3 grasses, salt, and gypsum treatments and 4 replications. Salt treatments include salinity level of 10 dS/m, 20 dS/m and non treated control. Gypsum treatments are 0, 11, and 22 g Ca /m^2 . The Irrigation water of different salinities were prepared by adding synthetic sea salt (Instant Ocean) to distilled water to obtain desired Electrical conductivity (EC) of 10 and 20 dS/m. Before salinity treatment ET was calculated by irrigating the pot, till the water was running from the drainage hole. After 12 hours the drainage was blocked since we want to accumulate the salt to see the affect. Based on the ET loss the pots were irrigated with salt water and 150 ppm N will be applied every two days along with irrigation water. To reduce the salt shock the EC was gradually increased every two days from 2, 4, 8, 10,15 and 20 respectively during first week and than grown under full salt conditions for one week and the top growth was clipped. After second week mitigation was applied to the pots by adding gypsum once in two weeks.

Irrigation was applied after calculating the weight loss through evapotranspiration using load cells, to measure the difference in weight. Gypsum was applied to pots by dissolving it in distilled water and using hand held pressure sprayer (2 Lit.) and walking around the treatments to apply uniform spray.

Data Collection

Turf quality ratings was recorded weekly based on color, texture, uniformity, and density of the surface. Quality was visually evaluated from 1-9, 1= brown dead turf, 7= acceptable turf, 9= ideal turf. Clippings were collected on weekly basis by placing the pots on a large cardboard sheet and cutting the top growth above the pot rim by using Black and Decker clippers. Clippings were then collected from the sheet into a paper bag and oven dried at 65^{o}C for 24h and dry weight was taken.

The water used for plant growth was calculated by measuring the lysimeter pots using load cells. Initially the pots were saturated 100% and allowed to drain for 12 hours. Then bottom drainage slot was blocked using ear plugs and initial weight was taken. Thereafter based on the reduction in weight (initial weight - present weight) the water was applied to the plots using ‘Dispensette’ for precise application of salt treatments.

Statistical Analysis

All the data obtained will be subjected to analysis of variance or regression analysis in JMP 10 (Version 10.0.1, SAS Institute, Cary, NC). The main effect of grass, salt and gypsum, and all interactions were examined. Non-significant terms were systematically removed from each model. Means were separated with Fisher’s LSD (α=0.05) when appropriate.

RESULT AND DISCUSSION

Turf quality (TQ) was found to be influenced by the salinity regime and grass genotype is presented in Table. 2.1. The averages of TQ were 8.35 for control and 6.93, 6.04 for salinity regimes. Salinity had significant effect on clipping yield ($p<0.0001$). The overall TQ remained above acceptable level 6 for all the grass genotypes. The genotype ‘Zeon’ and ‘TifEagle’ exhibited significant difference among them while ‘Platinum’ did not exhibit any significant difference with ‘Zeon’. The highest overall mean was shown by ‘Platinum’ (7.29) genotype followed by ‘Zeon’ (7.19) and ‘TifEagle’ (6.84). Over the course of five week study TQ changed due to salinity stress and genotype and can be seen in Figure 2.1 also no interaction effect was found between gypsum and grass at the conclusion of the study.

The results suggested that use of gypsum did not account for increase or decrease in TQ of the grass genotypes under salt stress. Similar results were found by St.John *et al.* (2001) on creeping bentgrass and Kentucky bluegrass. TifEagle showed acceptable TQ under salinity stress (Shahba, 2010) and Platinum and Zeon showed greater TQ compared to hybrid bermudagrass (Dudeck *et al.,* 1983; Sharon *et al.,* 1998).

Clipping yield was determined on weekly basis by drying the clippings for 24h at 65^{o}C. Grass and salinity significantly influenced the clipping yield of the grass genotypes during the study period. Salinity treatments significantly ($p<0.0001$) resulted in an average clipping weight of 0.019 g cm^{-2} for 10 dS m^{-1} and 0.016 g cm^{-2} for 20 dS m^{-1} compared to control salinity 0.026 g cm^{-2}.

Average clipping yield for ‘Zeon’ was 0.030 g cm^{-2} compared to 0.025 and 0.024 g cm^{-2} for ‘Platinum’ and ‘TifEagle’ (Table 2.2). Also a significant turfgrass species*salinity level interaction was noticed in the study (Figure 2.2). Reduction in biomass production of grass genotypes under salinity stress is more obvious (Pessarakli and Touchane, 2006). The decrease may be due to ion toxicity or imbalance, water potential difference and lower accumulation of carbon products (Greenway and Munns, 1980; Munns and Termatt, 1986). At higher salinity growth limitation can also be due to depletion of energy and loss of torgor (Marcum, 2006).

Gypsum treatment did not show any effect on the clipping yield of the grass genotypes. ‘Zeon’ exhibited higher clipping yield compared to ‘TifEagle’ and ‘Platinum’ at the conclusion of the study. Literature on vegetable research suggest that external application of calcium help to improve Ca content in leaves (Spiers, 1993; Spiers and Braswell, 1994) but did not improve color, plant health or quality by increased Ca. In contrast, the clipping yield was found to be improved under external Ca application for grasses grown on silica sand (St.John *et al.,* 2001).

Evapotranspiration (ET) was determined weekly based on the average of the week during the study period. ET was influenced by salinity and gypsum on grass genotypes. Salinity levels significantly affected the ET ($p<0.0001$); control had the highest ET of 0.80 mm followed by 10 dS m^{-1} and 20 dS m^{-1} at 0.58 mm and 0.41 mm respectively (Table 2.3). There was no difference between the control gypsum treatment (0.65 mm) and 11 g Ca m^{-2} (0.61 mm).

However, gypsum applied @ 22 g Ca m^{-2} showed significant difference (0.53 mm) from other treatments. Among the genotypes all the grasses showed significant differences ($p<0.001$), ‘Platinum’ recorded the highest ET of 0.78 mm followed by ‘TifEagle’ and ‘Zeon’ (Figure 2.3).

Sand has very low Cation Exchange Capacity (CEC), thereby limiting the amount of cations that they can hold at any time (Nelson, 1991). This suggests the significance of gypsum application on ET of the grass types in the study (Figure 2.4). Due to higher osmotic potential in the root zone the grass genotypes were unable to uptake the water. Thus reducing the water use with increase in salinity levels during the course of the study.

CONCLUSIONS

The examination of mitigation has been conducted in common warm season turfgrasses under salinity stress. Foliar application of gypsum did not show any significant effect on turf quality and clipping yield during the study period. However, ET showed significant difference for gypsum application to the grass genotypes. Platinum showed greater TQ (Marcum and Pessarakli, 2006) and ET values under salinity stress indicating that the genotype is able to perform well under high salinity level compared to other grass genotypes in the study. It was interesting to note that ‘Zeon’ had higher clipping yield production under salinity stress compared to TifEagle and Platinum. However, no significant difference was noticed in clipping weight performance for Platinum and TifEagle but both these genotype showed significant decrease in the TQ in the study. It is possible that Zeon was able to produce greater clipping yield under salinity stress with reduced TQ. Future research examining the performance of Zeon under various salt concentrations needs to be determined.

Among the interaction grass*salt showed significant difference for ET in the study. Although control and 11.0 g Ca m-2 application did not show any significant difference for ET between them. This might be because the additional gypsum did not affect the soil CEC thereby showing no significance. But gypsum applied @ 22.0 g Ca m^{-2}, there was significant change in ET for the grasses in the study. The ET reduced with increase in gypsum application as Ca from the treatment occupied the CEC of the sites. Which increased the osmotic potential of grass root zone to be higher compared to the osmotic potential inside the roots resulting in lower uptake of water by the grasses.

TABLES AND FIGURES

Table 2.1. Turf quality of ‘Zeon’ zoysiagrass, ‘TifEagle’ bermudagrass and ‘Platinum’ seashore paspalum as influenced by salinity regime, gypsum regime, genotype at the end of study period.

Main effects	**Turf quality**
Salinity (S) (dS/m)	
Control	8.35a
10	6.93b
20	6.04c
Gypsum Regime (g Ca /m^2)	
Control	7.18a
11 g	7.08a
22 g	7.05a
Genotype(G)	
Platinum	7.29a
Zeon	7.19a
TifEagle	6.84b
ANOVA	
Grass Salt Grass*Salt	*** *** ***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

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Table 2.2. Evapotranspiration of ‘Zeon’ zoysiagrass, ‘TifEagle’ bermudagrass and ‘Platinum’ seashore paspalum as influenced by salinity regime, gypsum regime, genotype at the end of study period.

Main effects	**Evapotranspiration (mm)**
Salinity (S) (dS/m)	
Control	0.80a
10	0.58b
20	0.41c
Gypsum Regime (g Ca /m^2)	
Control	0.65a
11 g	0.61a
22 g	0.53b
Genotype(G)	
Platinum	0.78a
Zeon	0.39b
TifEagle	0.62c
ANOVA	
Grass Salt Gypsum Grass*Salt	*** *** *** **

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table 2.3. Clipping weight of ‘Zeon’ zoysiagrass, ‘TifEagle’ bermudagrass and ‘Platinum’ seashore paspalum as influenced by salinity regime, gypsum regime, genotype at the end of study period.

Main effects	**Clipping yield (g/cm^2)**
Salinity (S) (dS/m)	
Control	0.026a
10	0.019b
20	0.016b
Gypsum Regime (g Ca /m^2)	
Control	0.021^{NS}
11 g	0.020
22 g	0.021
Genotype(G)	
Zeon	0.030a
Platinum	0.025b
TifEagle	0.024b
ANOVA	
Grass Salt Grass*Salt	** *** **

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

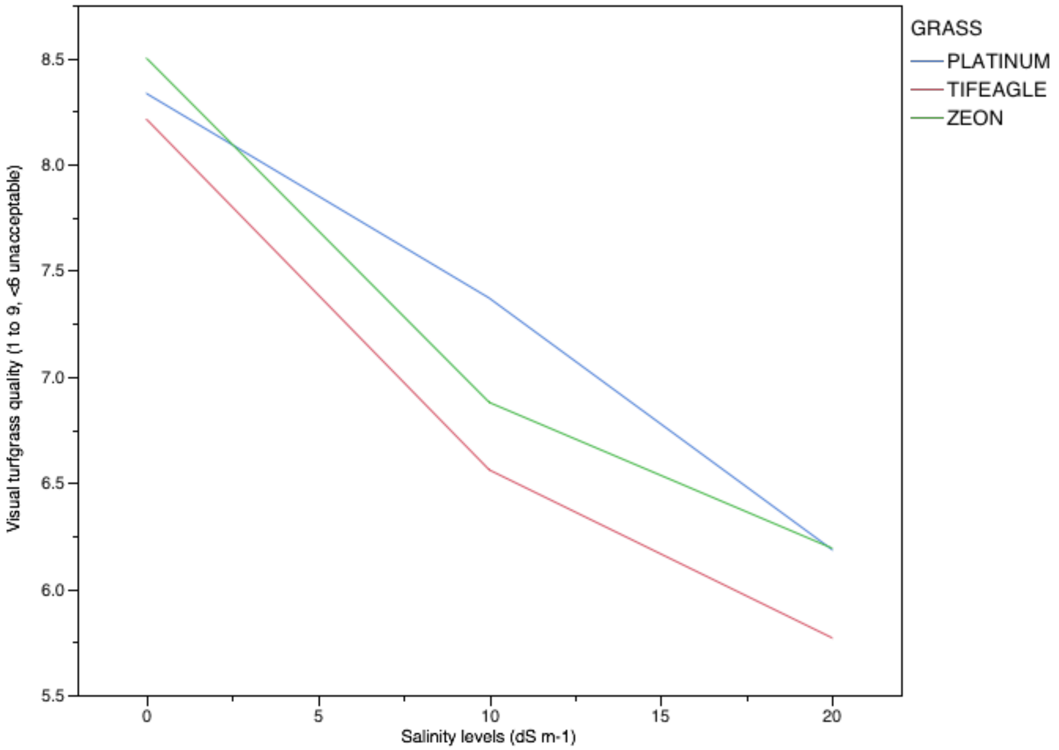


Figure 2.1. Turfgrass visual quality as affected by different salinity levels

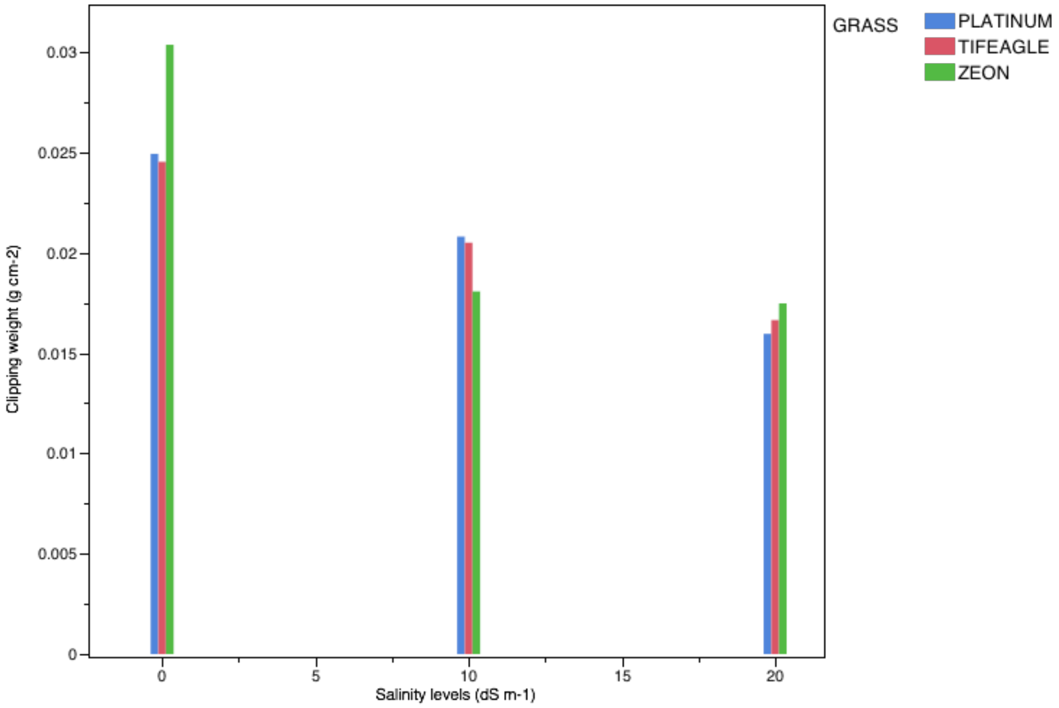


Figure 2.2. Clipping weight as affected by different salinity levels on turfgrasses.

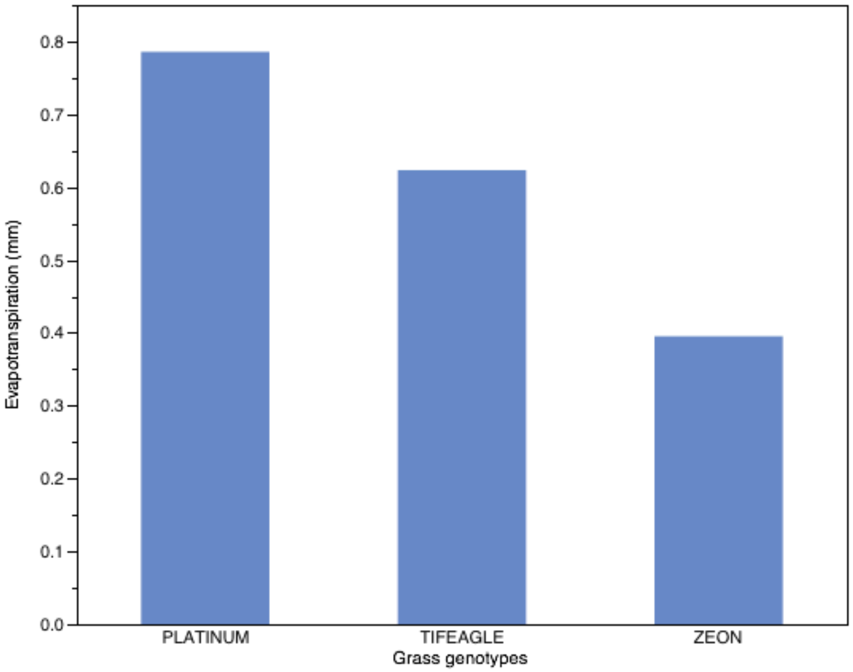


Figure 2.3. The interaction of salinity stress and gypsum regime on average evapotranspiration of the grasses in the study.

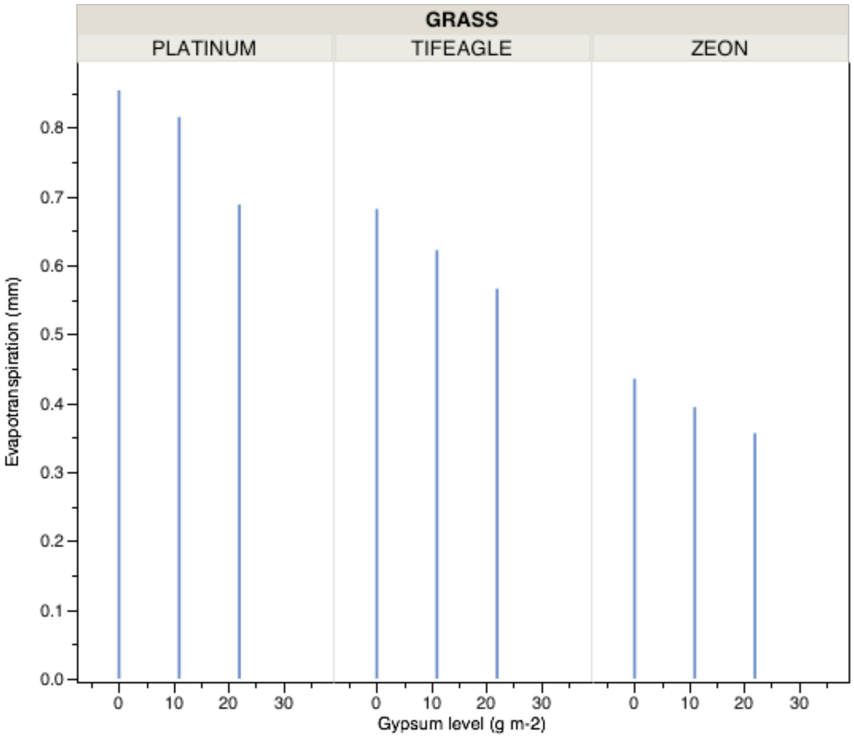


Figure 2.4. Evapotranspiration as affected by gypsum regime on turfgrasses.

Chapter Three: Effect of salinity and gypsum mitigation on warm-season turfgrasses

ABSTRACT

Salinity have been a major issue world wide for turfgrass manager who use effluent or reclaimed water for irrigation on turfgrass or in areas with natural sodic soils. It is essential to identify warm-season turfgrasses with increased salinity tolerance and to identify mitigation techniques applied to salt affected turfgrass sites, especially for sand based golf greens and athletic fields. A field screening method utilizing overhead irrigation was developed in India to mimic the challenges caused by saline water and for applying gypsum as a mitigation technique in the field. Zoysiagrass(*Zoysia matrella*), bermudagrass (*Cynodon dactylon x Cynodon transvaalensis*) and seashore paspalum (*Paspalum vaginatum. Swartz*) were planted in a sand rootzone arranged in a randomized complete block design with 3 replications and were irrigated with salt water (EC= 5, 10 and 15 dS m^{-1}) and followed by mitigation treat with gypsum application (23 g Ca m^{-2}). There were differences in salinity tolerance among the grass genotypes based on physiological, morphological and biochemical parameters. Seashore paspalum was the most salt tolerant followed by zoysiagrass The least salt tolerance was exhibited by bermudagrass. After the 6 weeks of initial salt treatment gypsum was applied and the visual turf quality, percent leaf firing, clipping weight and relative water content were measure weekly. The visual turf quality was reduced and the leaf firing increased as the salinity level increased. Electrolyte leakage and proline

increased under this stress conditions. Additional calcium from the gypsum application helped in maintaining membrane stability thereby reducing the electrolyte leakage. The level of chlorophyll that was degraded increase as the amount of salinity increased. Sodium content in root and shoot tissue were found to be irrespective of the grass and salinity while the K and Mg decreased with an increase in salinity levels. Calcium application affected the Ca content in root tissue. These results however indicate additional calcium does not help to improve growth, turf quality or clipping yield in turfgrass under salinity stress.

INTRODUCTION

Salinity is an important plant growth abiotic stress all over the world (Ashraf et al., 2008). Pessarakli and Szabolics (1999) reported that worldwide 100 M ha of land have become salt affected because of using saline irrigation water. A major problem with using saline irrigation water is decreased soil permeability (Carrow and Duncan, 1998) which results in decrease in soil oxygen, increase in water holding capacity, reduced pore space and increase in soil hardness (Pessarakli, 1994). The change is mainly due to the sodium (Na) ions, which dominates CEC (Cation exchange Capacity) causing dispersion (Bauder and Brock, 2001). But this might not be a problem on sand based root zones with low CEC (Christians, 1990).

In turfgrass sites, water related issues due to use of recycled water causes reduced growth, nutritional imbalance, ion toxicity and tissue dehydration (Katerji et al., 2000). Proper management and grass selection is important to tackle this situation. The stress caused by increased salt in irrigation water results in foliar burn thereby reducing the turf quality. Harivandi (2004) found that Ca ions were stripped from leaf tissues and often resulted in K leakage which in turn reduced the osmotic potential. Turfgrass canopy usually showed yellowing in older leaves exhibiting Ca deficiency (Duncan et al., 2000). The USGA (United States Golf Association) specification golf course putting greens utilize sand based root zones (Snow, 2003). Sand based root zones have high infiltration rate, limited compaction, and very good drainage. Additionally, sand based root zones have low CEC and also the sand can be calcareous or silica based. St.John et al. (2001) found that additional calcium was helpful for turfgrasses grown on silica based sand but not on calcareous sand. No study has been done to study the affect of calcium on salt affected turfgrass sites that are based on silica sand. Salinity screenings for turfgrass done most under green house conditions (Pessarakli and Kopec, 2009; Suplick-Ploense et al., 2002). Very few research has been published on warm season turfgrasses under salinity stress in open field conditions (Koch, 2012). Three warm season turfgrasses examined in this study: bermudagrass, seashore paspalum, and zoysiagrass, were chosen based on their salinity tolerance mechanism, wide spread nature and overall performance (Dudeck et al., 1983; Dudeck and Peacock; 1985 Carrow and Duncan, 1998; Marcum, 2008b).

Due to the popularity of gypsum as a calcium source to mitigate the salt stress in turfgrass management, this study was done to evaluate if the application of gypsum on sand based turfgrass under salinity stress would significantly influenced warm season turfgrass performance and physiology. The objectives of the study were 1) to identify the physiological changes in each grass genotype in response to salinity 2) to study the nutrient status of three popular warm-season putting green turfgrasses in response to salinity and 3) to determine the effect of gypsum application on grasses growth and nutrient status under salinity stress.

MATERIALS AND METHODS

Field Preparation and Planting

This study was taken up at the Botanical Garden, Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India during the year 2014. The experimental site was prepared by removing the top soil to a depth of 45 cm and filled with two different layers. The bottom layer was lined with granite pebbles of 0.4-1cm diameter and top 30 cm was filled uniformly with sand. The physical, chemical, and size distribution of the sand media are displayed in Appendix A-1 and A-2. Plots of 1.2 x 1.2 m^2 were formed and differentiate between plots hollow blocks were used. The zoysiagrass was established through sprigs collected from Turfgrass Research Facility in Department of Floriculture and Landscaping on January 24, 2014 and ‘Tif’ series bermudagrass was procured from ‘Coimbatore Golf Club’ and established through sprigging method at the research plot on January 25, 2014. Seashore paspalum was brought in from Rakindo Golf, Coimbatore and sprigged at the experimental plot on March 11, 2014. The roots were washed free of soil before planting them in the research plot. Each plot contained 25 sprigs planted at a spacing of 10 cm^2. A total of 6 replications of each grass genotypes were planted in a randomized block design

Plant culture and Treatment Application

During planting the plots were fertilized with 90 Kg N Ha^{-1} and fresh water irrigation was applied as needed. Throughout the growing season foliar application of nitrogen was applied at the rate of 4 g N m^{-2} with a CO_2 pressurized back pack sprayer with a ASPEE 5008 nozzle. The grasses were mowed weekly at 3.5 cm with clippings removed and pest incidence of aphids and thrips were taken care by timely application of imidacloprid @ 1 ml in 3-4 liters of water. After establishment, grasses were irrigated with saline solution made of Na Cl salts. The salt solutions were made to an Electrical Conductivity (EC) of 5, 10 and 15 dS m^{-1} and was made in a 200 liter tank each with a mechanical agitator and electricity powered pump was attached. The pump and tanks were connected through hoses and each plot were irrigated with 10 liters of the salt solution daily for the 12 week of study period. This amount of irrigation resulted in 6.94 mm. Applications began on 28 July 2014 with final application on 20 October 2014. Throughout the experiment untreated control was maintained for each grass genotype that were irrigated with 10 L of water without any salt.

Following the salinity regime for 6 weeks, gypsum was applied at a rate of 23 g Ca m^{-2} by dissolving it in water and applying using ASPEE backpack sprayer at biweekly intervals to half of the replicates. Applications were carried out on 8 Sept, 22 Sept, 16 Oct, and 20 Oct 2014. The salinity treatment was continued until the end of experiment. This study was a randomized block design with three replications. Treatments included three salinity levels (5, 10, and 15 dS m^{-1}), salinity with gypsum, and a non-treated control in which the plots received salinity treatment but not the additional gypsum application.

Data Collection and Analysis

The following parameters were measured: turf quality (TQ), leaf firing (LF), relative water content (RWC), clipping weight (CW), root length (RL), electrolyte leakage (EL), proline content, and plant tissue nutrient analysis. On a weekly basis TQ was rated after gypsum treatments were initiated. TQ is based on a combination of factors composing of color, density, uniformity, and texture. TQ was evaluated on a scale of 1 to 9 where 1= brown or dead turfgrass, 6= minimum acceptable turfgrass, 9= perfect turfgrass condition (Turgeon, 2011). Per cent LF was taken weekly for all plots to estimate the leaf senescence due to salinity stress and mitigation. Clippings were collected weekly by cutting the grass at 5 cm height and dried for 48h at 65°C, and weighed. For leaf RWC analysis, 100 mg of leaf samples were clipped and weighed (W). The clippings were then put into petri dish with de-ionized water for 24 h at 4°C. The leaf tissue were then removed from the water bath, dried and weighed for obtaining turgid weight (TW). Leaf clippings were then dried in oven at 80°C for 72 h and weighed (DW) (Barrs and Weatherly, 1962). RWC is then calculated by using the equation RWC (%) = [(W-DW) / (TW-DW)] x 100 (Barrs and Weatherly, 1962).

To estimate the membrane integrity, leaf electrolyte leakage (EL) was determined. First, 0.5 g fresh leaf tissue was taken and rinsed using Millipore water to ensure that excess salt, fertilizer and gypsum residue were removed. Next, 20 ml of deionized water added and the tubes were incubated for 24 h at 4°C. Following the incubation, initial conductance (Ci) was measured with conductance meter after which the leaves were autoclaved for 50 minutes and Millipore water was made up to the original level before autoclave and conductance was measured (Cmax). The relative EL was estimated by using the equation (Ci/Cmax) x 100 (Blum and Ebercon, 1981).

Proline content was analyzed from 100 mg leaf sample according to Bates (1973) utilizing spectrophotometer at 520 nm. The chlorophyll content was measured biweekly using the formula mg/g= ([20.2 (OD @ 645 nm) + (8.02) (OD @ 663 nm)] x V) / (fw x 1000), according to the method of Yoshida *et al.* (1971). At the end of the treatment period plugs were taken from the research plot using core sampler of 10 x 20 cm and the sand from root zone media was gently shaken from the root sample and length were measured using a meter scale.

The root and shoot samples were dried in oven and 100 mg of dried sample were place in 100 ml digestion flask and 5 ml of triple acid extract (HNO_3: H_2S0_4 : H_2O_2) was added to each flask. The flasks were then digested over sand bath at 200^0C until a clear solution was obtained. After digestion the flasks were removed from the digestion plate and cooled to room temperature. The volume in each flask was adjusted up to 100 ml using distilled water. The samples were analyzed for Na^+, K^+, Ca^{++}, and Mg^{++} by Atomic Absorption Spectrophotometer expressed as mg g^{-1}.

Statistical Analysis

The JMP pro (version 10.0.0; SAS Institute, Cary, NC) Software package was used for the data analysis. Main effects include salinity levels, gypsum, and time in weeks. All possible interactions of main effects were evaluated. Non-significant terms were systematically removed from each model from highest order of non-significant interactions. Means were separated with Fisher's protected LSD ($\alpha = 0.05$) when applicable.

RESULTS

By the end of July the grasses established uniformly and the initial salt treatments began on 28 July, 2014. After 6 weeks of salinity treatment, gypsum application was done on biweekly basis. The gypsum did not have any effect on the visual turfgrass quality (Table 3.1). The turfgrass quality rating was lower as the experiment progressed, independent of gypsum treatment indicating that the grasses were experiencing salinity stress. Leaf firing was the major factor leading in reduction of turf quality and several plots of bermudagrass experienced severe quality loss.

There was a significant Grass x Salt interaction for visual turfgrass quality scores (Table 3.1). Turf quality scores were highest for seashore paspalum grass among all the grasses. All the grass controls did not show any sign of stress for the treatment period of 6 weeks with visual turf quality above 8.0 (Appendix A-4). The gypsum control and salt control had similar turf quality throughout the experiment indicating no affect of gypsum on salinity stress. Also there was no significant difference between the gypsum treatments suggesting that gypsum was not able to regulate the turf quality of grasses under salinity stress grown of sand.

A significant Grass x Salt x Week interaction occurred for turf quality in the study (Table 3.1). Increase in the duration of salinity the turfgrasses were exposed to the turf quality reduced. The visual turf quality decreased with increase in salt concentration irrespective of the grass genotype (Appendix A-4). The salinity and week also had significant interaction on visual turf quality which was evident throughout the study. (Table 3.1).

Leaf firing was significantly reduced under Grass x Salt x Week interaction (Table 3.1). The seashore paspalum had the lowest leaf firing compared to bermudagrass and zoysiagrass (Appendix A-4). The salinity control and gypsum control had similar no leaf firing during the entire study period. A significance at (0.05) was noticed for Salt x Gypsum after 3 weeks which later subsided. This might be due to significant effect of gypsum on electrolyte leakage (Table 3.2). Reduced leakage may contribute towards the reduction of leaf firing in grasses.

Relative water content was affected by salt treatment (Appendix A-6). The gypsum treatment did not influence the relative water content of the grass genotypes. The gypsum control and salinity control had similar relative water content throughout the study period implementing no significance. The relative water content showed significance in consecutive weeks with increase in salinity (Table 3.1). Seashore paspalum grass had greater relative water content followed by zoysiagrass and bermudagrass.

Clipping weight was affected by salinity levels (Appendix A-7). The salinity reduced the clipping yield significantly while gypsum application did not stop the reduction in clipping weight. Clipping weight was greatest at the beginning and in the progressing weeks the clipping weight reduced. A significant interaction between salinity x week was noticed in the study (Table 3.1). The seashore paspalum recorded the lowest reduction of clipping yield among all the grass genotypes (Appendix A-7).

Electrolyte leakage increased with increase in salinity in the study (Appendix A-8). Gypsum treatment significantly reduced the EL in grasses compared to non-treated grass genotypes. After 6 weeks of gypsum treatment significant reduction in leaf firing was noticed for grasses exposed to salinity (Appendix A-8). A significant interaction between Grass x Week x Salinity x Gypsum was noticed in the study (Table 3.2).

Proline content increased with increase in salinity and gypsum treatment did not have any significant affect on proline content in leaf tissue (Table 3.2). There was significant Salt x Week interaction for proline accumulation in shoot tissue (Appendix A-9). Seashore paspalum accumulated the least amount of proline among all the grass genotypes.

Chlorophyll content changed from 2, 4 and 6 weeks of the gypsum application (Appendix A-10). The chlorophyll content decreased with increase in salinity for different grass genotypes. A significant Grass x Gypsum x Week interaction was found in the study (Table 3.2). This might be due to reduction in EL after gypsum treatment which might have reduced the leaf firing to some extent (Table 3.1, 3.2).

There was significant Grass x Salt interaction for root Ca and Na in the study except for Mg and K while Ca and K were significant for gypsum treatment Mg and Na were non significant (Appendix A-11). Bermudagrass had highest Ca and Na in root tissues compared to other grass genotypes. Seashore paspalum showed significant amount of Mg and K in the root tissue compared to other grasses. A significant Grass x Gypsum and Salt x Gypsum was noticed only for Ca in the study (Appendix A-11). Salinity had significant effect on root length of the grass genotypes. There was significant Grass x Salt effect for root length in the study. Gypsum treatment did not have any significant effect during the study period. The root lengths were longer at 5 dS m^{-1} salt treatment compared to control but with increase in salinity the root length decreased. Seashore paspalum recorded the lowest root length among the grass genotypes in the study (Appendix A-11).

A significant Grass x Salt interaction was found for shoot Ca, Mg, and Na in the study while K was found to be non significant (Appendix A-12). Only Ca was found to be significant for gypsum treatment in the study. Also Mg was found to be significant for Grass x Gypsum interaction (0.05) and K for Grass x

Salt x Gypsum interaction. Control salinity recorded the highest Ca, Mg, and K content in shoot tissue. Seashore paspalum recorded the highest nutrient content in shoot tissue compared to other grass genotypes.

DISCUSSION

Grass response to salinity stress mitigated with additional calcium

Salinity irrigation treatment resulted in significant differences in the grass genotypes reponses. It resulted in decreased turf quality (Figure 3.1), clipping weight (Figure 3.2), relative water content (Figure 3.3), electrolyte leakage (Figure 3.4), and chlorophyll content (Figure 3.5). These results are coincided with other salinity tolerance research by (Koch and Boncos, 2010; Pessarakli and Kopec, 2008; Dai *et al.,* 2008; Lee et al., 2005; Qian *et al.,* 2004), who also found similar pattern of reduction in parameters with increased salinity treatments.

Seashore paspalum exhibited the highest relative water content chlorophyll content, and clipping yield thereby was able to suffer less reduction in growth compared to bermuda grass that exhibited greater reduction in growth under salt stress. These results are in par with (Pessarakli et al., 2008; Bauer et al., 2009; Marcum and Murdoch, 1990; Marcum and Pessarakli, 2006).

The different salinity levels resulted in significant reductions in most of the parameters independent of gypsum treatment suggesting the effect due to stress caused by salinity. Furthermore, visual turf quality reduced drastically when grass genotypes were irrigated with different salinity levels. Longer the grass exposed to salinity the lower the turf quality score (Figure 3.6). Higher concentration of sodium exposed to grass is known to cause leaf firing and reduction in turf quality (Dudeck and Peacock, 1984). Salinity reduces visual turf quality and leads to increased leaf firing (Qian and Meccham, 2005). Seashore paspalum and zoysia grass were able to maintain turf quality above the acceptable score (6) and bermuda grass turf score fell below the acceptable level at the end of the study. Gypsum treatment did not have any significant effect on turf quality (Figure B-1). Similar results were found by Spiers (1993) on vegetable crops where additional Ca did not improve plant health, growth or color.

Salt stress injures to the canopy occur as the higher concentration of NaCl turns the canopy color to light green to brown (Pessarkli and Kopec, 2008). Salinity increased leaf firing in the turfgrasses during the study (Figure 3.7) and gypsum did not have any significant affect on leaf firing (Appendix B-2). Leaf firing is closely related to salinity tolerance of the grass (Uddin and Juraimi, 2013). Compared to their respective controls, the increase in leaf firing and decrease in turf quality were more for bermudagrass followed by zoysiagrass and seashore paspalum.

Bermudagrass has been shown to have the least tolerant to salinity with the highest leaf firing (Dudeck *et al.,* 1983) followed by zoysia grass (Qian *et al.,* 2001) and the most tolerant of the turfgrasses being seashore paspalum (Shahba *et al.,* 2010). Leaf firing can be correlated with turf quality of the turfgrasses. In this study, increase in leaf firing resulted in decrease of visual turf quality (Appendix B-3). Qian and Meccham (2005) found similar pattern in turf quality and leaf firing in their studies.

Even though irrigateion was provided in excess of field capacity, significant difference in water status was noticed in the study. Higher water status within leaves is related to salinity tolerance (Alshammary *et al.,* 2004). In this study bermudagrass showed more severe decline in relative water content (RWC) compared to zoysiagrass and seashore paspalum under increased salinity levels (Figure 3.8). The available water to the grass could have been reduced at higher salt concentrations resulting in decrease of RWC in grasses (White *et al.,* 2001). Compared to the control, seashore paspalum and zoysia grass lost relatively less water suggesting that they are more salt tolerant compared to bermudagrass. The superior salt tolerance in seashore paspalum might be because of its ability to adjust stomatal opening at higher concentrations of salt (Liu *et al.,* 2011).

Munns and Tester (2008) suggested that the response of a plant to salinity stress in mainly due to osmotic changing phase and ion specific phase, both of them have significant effect on water uptake or status of the plant. Salinity causes osmotic stress causing decrease in cell water content resulting in turgor loss leading to reduction in RWC (Morant-Manceau *et al.,* 2004). The results observed in the study are in corroboration with that of Lee *et al.,* (2004).

Clipping weight are also used to quantify the salinity stress tolerance of a grass genotype (Marcum, 2001). Seashore paspalum showed the highest clipping yield suggesting that it is more tolerant to salinity compared to other grass genotypes in the study (Figure 3.2). The increase in salinity corresponded with decrease in clipping weight of the grass. These results are consistent with the research done by (Marcum and Pessarkli, 2006; Qian *et al.,* 2001; Dudeck *et al.,* 1983).

Chlorophyll content is an indirect measure of photochemical efficiency of photosystem II (Zhang *et al.,* 2003). Salinity tolerant cultivars should be able to maintain higher photochemical efficiency thereby high chlorophyll content. Seashore paspalum recorded the lowest chlorophyll reduction compared to bermuda and zoysiagrass (Appendix B-4). Gypsum application had significant affect on the chlorophyll content in the leaves of three warm-season turfgrasses with increase in duration of salt exposure (Figure 3.9). In comparison to control, bermudagrass had a steep decline in chlorophyll content indicating it to be the least salt tolerant of the grass genotypes studied. Increase in salinity stress significantly reduced the clipping yield and chlorophyll content. Stomatal opening and non-stomatal process involved in reduction of RWC (Berndt, 2007). Increase in salinity damaged the cell membranes in turfgrasses resulting in decreased turf quality and increased leaf firing in association with lipid peroxidation (Huang *et al.,* 2001).

In many parts of the world, salinity stress is found along with drought stress. Grass genotypes are found to be able to maintain root production under stress conditions (Alshammary *et al.,* 2004). Turfgrass can adapt to intermediate saline environment by increasing the root length (Ackerson and

Younger, 1975). Similar results were found in the study where at (5 dS m^{-1}) root length of the grass genotypes increased compared to the control. Zoysiagrass showed the highest difference in comparison to control but with increase in salinity root length dropped significantly for zoysia as well as bermudagrass (Dudeck et al., 1983;Marcum and Murdoch, 1990). Seashore paspalum was able to maintain relative root length even at higher salt concentration (Marcum and Murdoch, 1990). However, in salt sensitive species the root length decreased with increase in salinity as shown by zoysia and bermudagrass (Figure 3.10). Even though at higher salinity the root length was greater for plants treated with gypsum, the effect was not significant. Further studies will be helpful to determine this.

Salinity can damage cellular membranes and lead to chlorophyll degradation (Brown, 1982). The damage is quantified by electrolyte leakage, which is commonly used to measure heat, drought and salinity stress (Hodgkinson and Mackley, 1995). Electrolyte leakage decreased with application of gypsum (Figure 3.11). Gypsum treatment had significant effect on salinity stresses after 2 weeks of treatment (Figure 3.12). Seashore paspalum demonstrated least leakage at high salinity levels (Figure 3.4). Salt tolerant turfgrasses has strong membrane integrity, which prevents osmolytes from leaking out (Alarcon *et al.,* 1993). This might contribute to its superior salt tolerance along with higher RWC, Cw, chlorophyll content and lower electrolyte leakage (Liu *et al.,* 2011). Plants tolerate stress by osmotic adjustment (Smith *et al.,* 1989). Additional calcium in found to reduce the electrolyte leakage in the study. This is in corroboration with the studies done by (Cooke *et al.,* 1986; Coria *et al.,* 1998).

Calcium application is found to inhibit loss of chlorophyll under heat stress by reducing photo oxidation or by enhanced membrane integrity (Wise and Naylor, 1987; Coria *et al.,* 1998). External Calcium is found to increase salinity tolerance in bean (*Phaseolus vulgaris* L.) roots (Cachorro *et al.,* 1993). Jiang and Huang (2001) found that the osmotic adjustment increased during short term stress but external application of calcium did not effect the osmotic adjustment.

The additional calcium not only affects the membrane stability, but is also involved in regulation of antioxidant enzyme and oxidative signal transduction (MsAinsh *et al.,* 1996; Gong *et al.,* 1997). Thus, further long term studies should be done to determine the effect of external calcium on membrane integrity and salinity tolerance in plants.

Proline is one of the most common biochemical indicator to assess salinity tolerance (Chen *et al.,* 2001). Accumulation of proline is observed in plants under stress as a protection to stabilize the structure of proteins and membranes (Szabados and Savoure, 2010; Phang et al., 2008). Under saline conditions, the amount of proline is found to increase in turfgrasses (Mattioli *et al.,* 2009; Huimin et al., 2001), similar to the one observed in this study.

The addition of salt increased proline accumulation in leaves of bermudagrass, zoysiagrass and seashore paspalum over the course of the study (Figure 3.13). Similar results have been observed in turfgrasses

(Borowski, 2008; Huimin *et al.,* 2001). Hadam and Wronchna (2012) found increased proline content in perennial ryegrass and smooth-meadow grass with increase in salt concentrations. Under highest salinity level seashore paspalum showed the least amount of proline compared to bermudagrass and zoysiagrass (Figure 3.14). Bermudagrass accumulated the highest proline content compared to control treatment, this species is unable to defend itself from salinity stress (Marcum, 2002). The additional calcium did not have any significant effect on proline in the turfgrasses studied.

Seashore paspalum did not have more proline in relation to the control but it showed an increasing trend in proline contents in the study. This might be because of proline acting as a carbon and nitrogen sink for recovery as well as being the primary compatible osmolytes for osmotic adjustment (Ashraf and Foolad, 2007). Proline also functions as stabilizer of membrane and protein structure and buffers redox potential at cellular level during stress (Bartles and Sunkar, 2005; Ashraf and Foolad, 2007).

Impact of additional calcium on ion accumulation in turfgrasses

Salinity tolerance is related with high levels of K and Ca, as these ions help in maintaining cell membrane/wall integrity and controls turgor pressure (Flowers and Yeo, 1986; Lee *et al.,* 2007). Seashore paspalum maintained higher levels of K and Ca in this study (Appendix A-12), implying paspalum to be the salt tolerant grass among the different genotypes studied. Increasing salinity decreased shoot Ca concentration respectively (Figure 3.15). This might be because of replacement of water soluble parts of these elements by

Na as cellular osmolytes (Lee *et al.,* 2001). Additional calcium significantly increased the calcium content in the shoot tissue (Appendix A-12). Significant difference between grasses for Ca and Na is noticed in the study. Mg was highly significant for the grass genotypes (Appendix B-5). Irrespective of the salinity, Mg was higher than Ca for all the turfgrasses in the study.

Maintaining higher K content in shoot tissue contributes towards salinity tolerance of grass species (Colmer et al., 1995). Seashore paspalum had higher K content compared to other turfgrasses in the study (Appendix B-6). This is in par with studies done by (Dudeck and Peacock, 1985; Marcum and Murdoch, 1990). High sodium accumulation with increase in salinity corresponding with increased accumulation in salinity tolerant seashore paspalum compared to other grasses was noticed. Na uptake enhances the water potential gradient between grass and substrate thereby helping to maintain turgor pressure for shoot growth (Reimann and Breckle, 1993).

Correlation between salinity levels and K/Na ratio in shoots of the grass genotypes studied were not significant, suggesting that the different genotypes managed to maintain greater K concentration in shoot despite the salinity levels imposed on them (Figure 3.16). This result is similar to the results observed by (Uddin *et al.,* 2012).

On the root plasma membrane Mg competes with Ca for the binding sites (Grattan and Grieve, 1999). Salinity had significant affect on the Ca concentration in roots of grass genotypes in the study (Figure 3.17). Additional Ca in the root zone definitely had significant effect on salinity as well as grass genotypes for root length (Appendix B-7, Figure 3.18, 3.19). No obvious effects of Mg uptake on salinity tolerance was noticed in the turfgrasses in the study (Appendix B-8). Na content in roots increased with increase in salinity (Figure 3.20). The percent increase compared to control salinity was greater for Na (Appendix B-9). The concentrations of Mg and K decreased with increase in salinity levels.

There was no significant effect for turfgrass and salinity for K and Mg in the study. High external Na would usually affect the K in the root zone due to similar physiochemical properties (Maathuis and Amtmann, 1999). The non selective cation channels allows both K and Na to enter and also there are selective transporters like KUP (potassium uptake permease), HKT_1 (high potassium transporters) to transport Na and K into the turfgrass (Uddin *et al.,* 2012). But by addition of Ca in the root zone the Na is replaced thereby reducing the Na uptake and increase in K uptake which in turn causes non significant correlation between salinity levels and K/Na ratio in roots (Amtmann and Sanders, 1999). Which was observed in this study (Figure 3.21). Further studies is recommended to understand the effect of additional calcium on ion accumulation in turfgrasses.

CONCLUSIONS

Salinity treatment significantly affected the growth and quality of the turfgrasses studied. Additional gypsum in a sand based system did not increase turfgrass quality, clipping weight, chlorophyll content, leaf firing, and relative water content. Salinity treatment increased electrolyte leakage but calcium treatment reduced leakage after the second week of treatment. Increase in proline content suggests that the plants were responding to stressful conditions. Increased K and Na levels were observed in turf tissue as salinity stressed increased. Mg and Ca decreased with increase in salinity. Additional calcium exhibited amplified interaction between grass sand salinity levels. Further research is needed to investigate the mitigation effect involved.

Tables and Figures

Table 3.1. Analysis of variance of salinity tolerance and gypsum mitigation measurements on turf quality (TQ), leaf firing (LF), relative water content (RWC), and clipping weight (CW) evaluated in field conditions at salinity treatments (0, 5, 10, 15 dS m^{-1}) for zoysiagrass, bermudagrass and seashore paspalum over 6 weeks.

Source	df	P- value			
		TQ	LF	RWC	CW
Grass (G)	2	**	NS	***	***
Salt (S)	3	***	***	***	***
Gypsum (M)	1	NS	NS	NS	NS
Week (W)	5	NS	NS	NS	NS
G*S	6	***	***	***	***
G*M	2	NS	NS	NS	NS
G*W	10	NS	NS	NS	NS
S*M	3	NS	*	NS	NS
M*W	5	NS	NS	NS	NS
S*W	15	***	***	***	***
G*S*M	6	NS	NS	NS	NS
G*S*W	30	**	***	*	NS
S*M*W	15	NS	NS	NS	NS
G*W*M	10	NS	NS	NS	NS
G*S*W*M	30	NS	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level

Table. 3.2. Summary of repeated measures ANOVA table of main effects and their interactions on zoysiagrass, bermudagrass and seashore paspalum performance under salinity and gypsum regime.

Source	**df**	**F- ratio**		
		Electrolyte Leakage	**Proline content**	**Chlorophyll Content**
Grass (G)	2	0.42	17.55***	270.98***
Salt (S)	3	548.53***	1535.96***	633.12***
Gypsum (M)	1	0.17	1.27	0.11
Week (W)	2	0.52	0.58	0.13
G*S	6	59.00***	115.61***	139.13***
G*M	2	0.03	0.58	0.00
G*W	4	0.49	0.27	0.06
S*M	3	16.44***	1.46	0.44
S*W	6	10.79***	2.24*	11.35***
M*W	2	0.19	0.10	0.05
G*S*M	6	2.14	0.98	1.42
G*S*W	12	1.14	1.96*	3.55
S*M*W	6	4.44**	0.81	0.33
G*M*W	4	0.13	0.09	0.12**
G*S*M*W	12	1.84*	0.66	0.75

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level

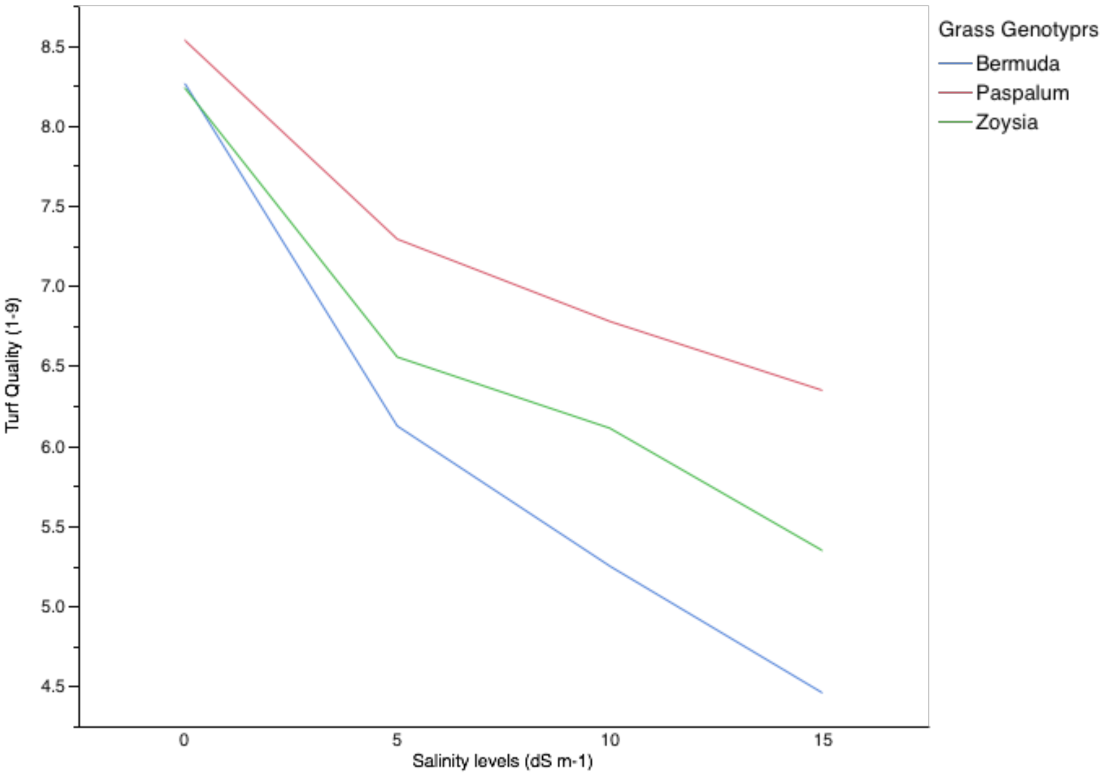


Figure 3.1. Turf quality of three warm-season turfgrasses as influenced by different salt regimes

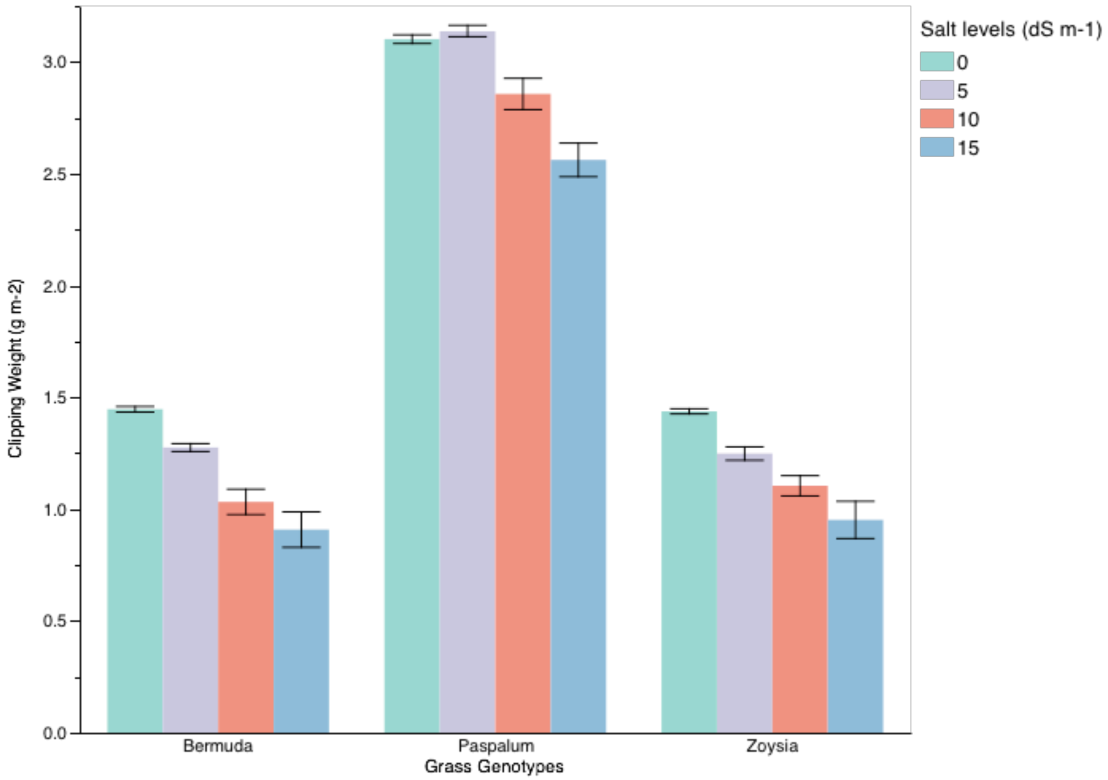


Figure 3.2. Clipping weight of three warm-season turfgrasses as influenced by different salt regimes. Means were separated at P≤0.05 by protected LSD.

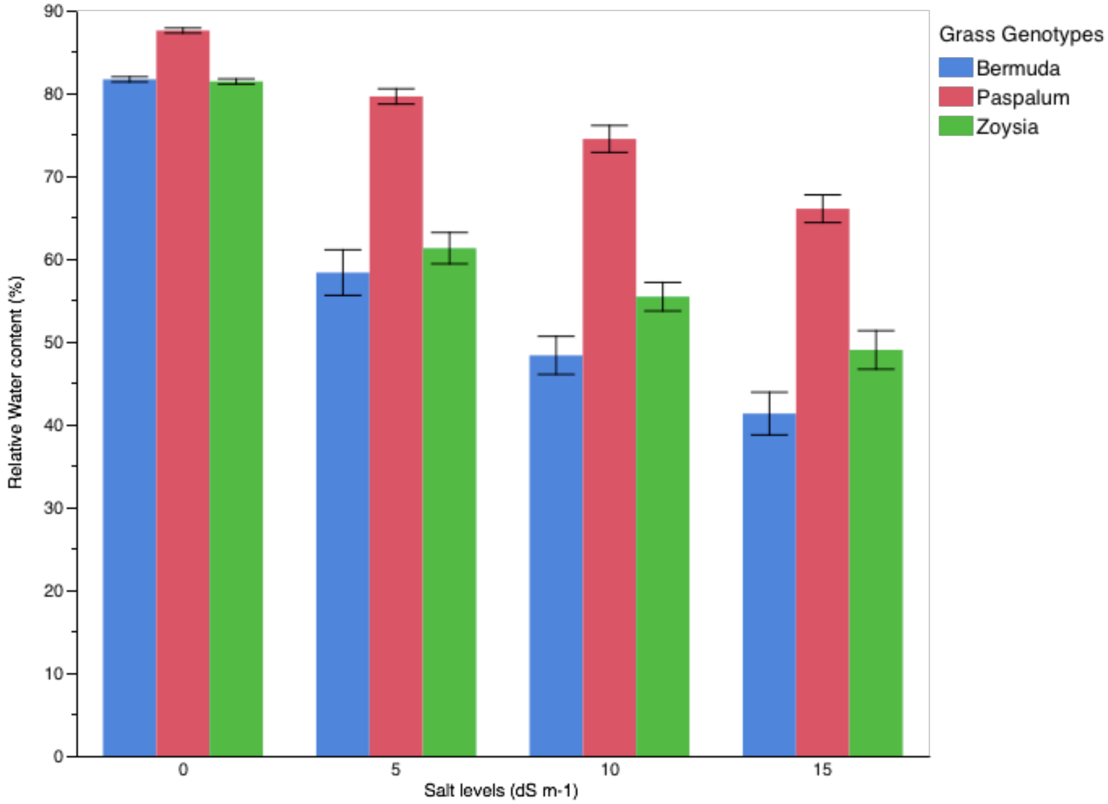


Figure 3.3. The effect of salinity on relative water content of three warm-season turfgrasses. Means were separated at P≤0.05 by protected LSD.

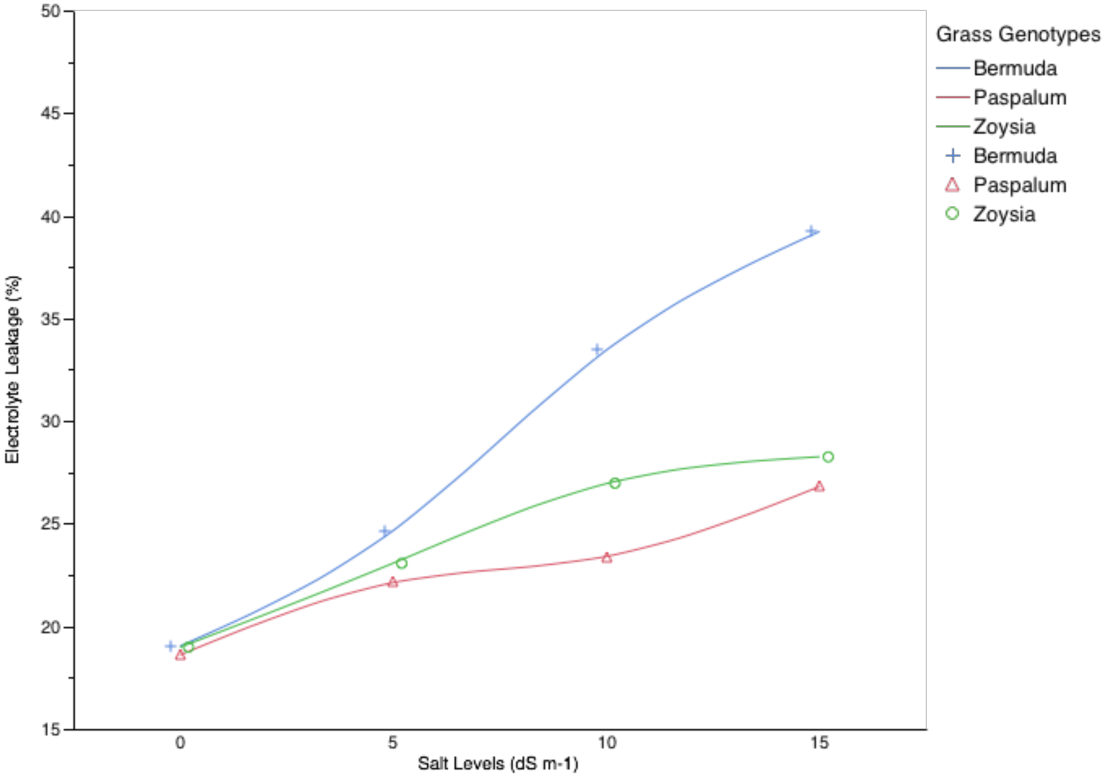


Figure 3.4. Electrolyte leakage of three warm-season turfgrasses as influenced by different salt regimes

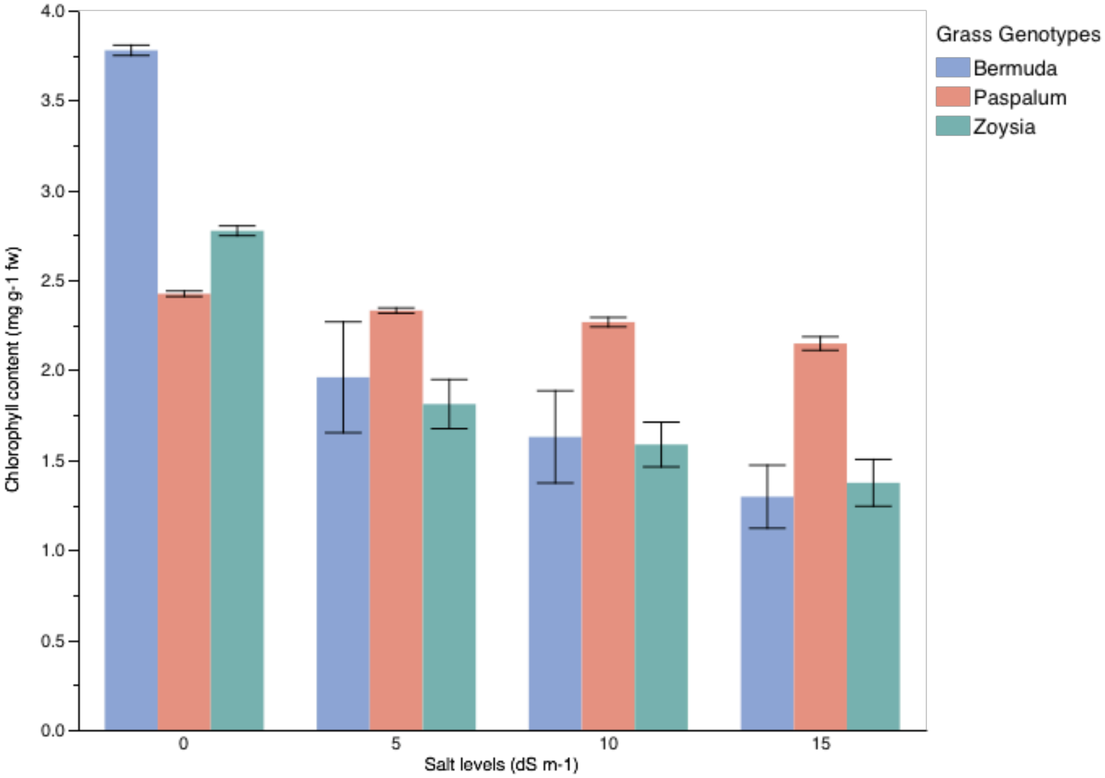


Figure 3.5. Chlorophyll content of three warm-season turfgrasses as influenced by different salt regimes. Means were separated at P≤0.05 by protected LSD.

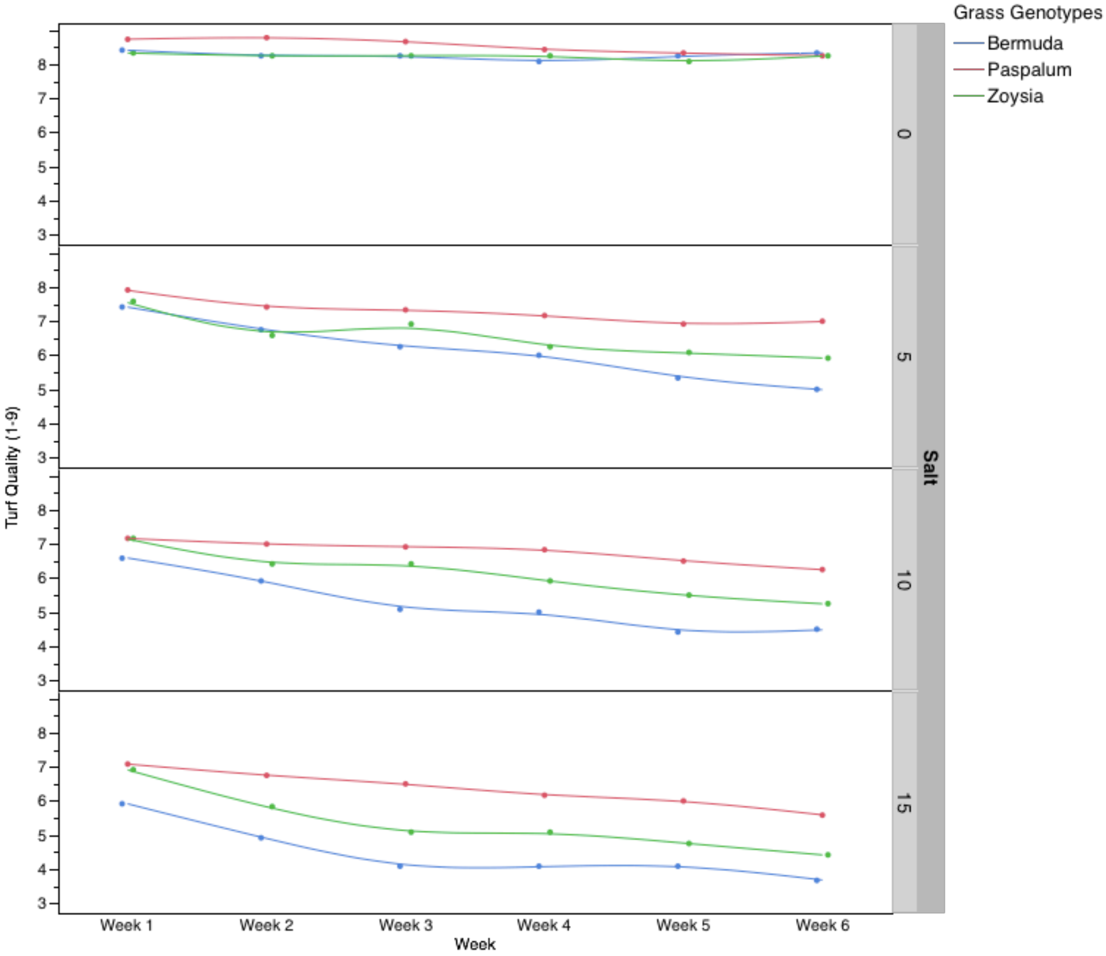


Figure 3.6. Visual turfgrass quality of three warm-season turfgrasses as influenced by salinity over the course of 6 weeks

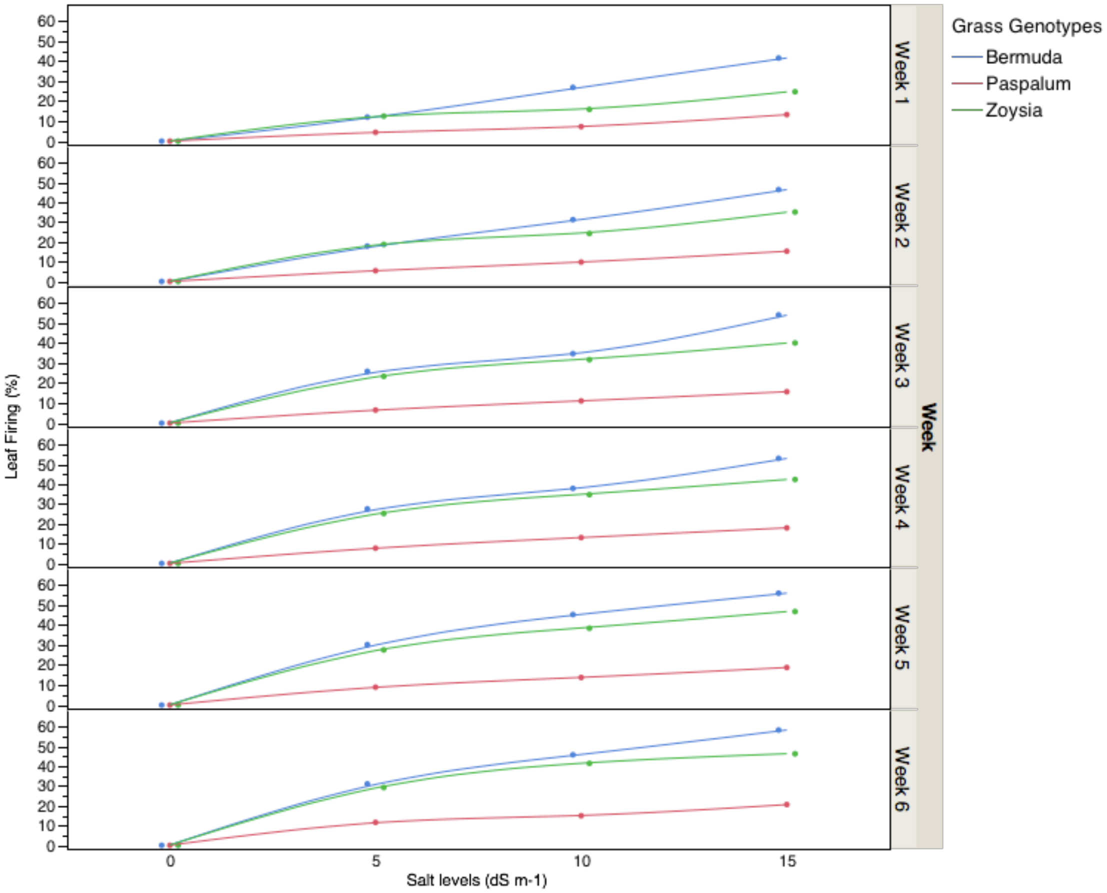


Figure 3.7. Leaf firing of three warm-season turfgrasses as influenced by salinity over the course of 6 weeks

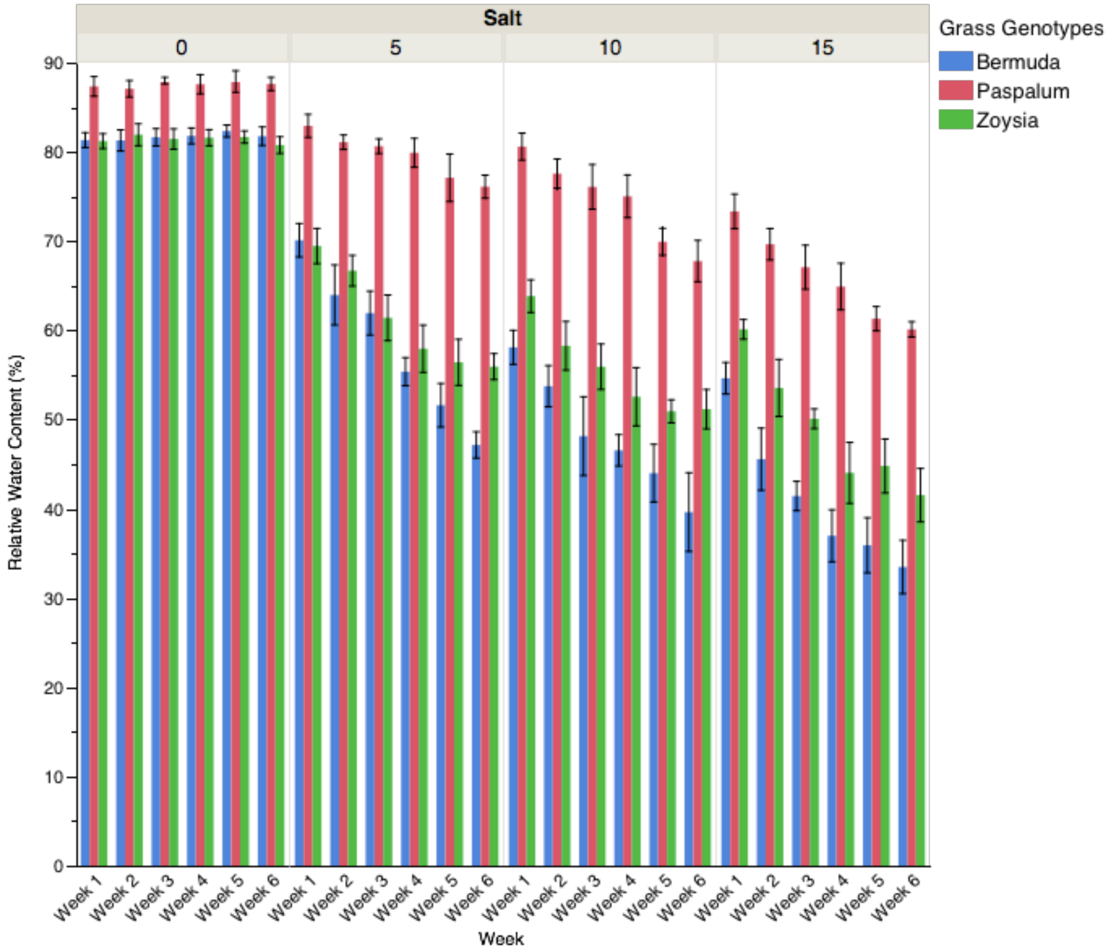


Figure 3.8. Relative water content of three warm-season turfgrasses as influenced by salinity over the course of 6 weeks. Means were separated at P≤0.05 by protected LSD.

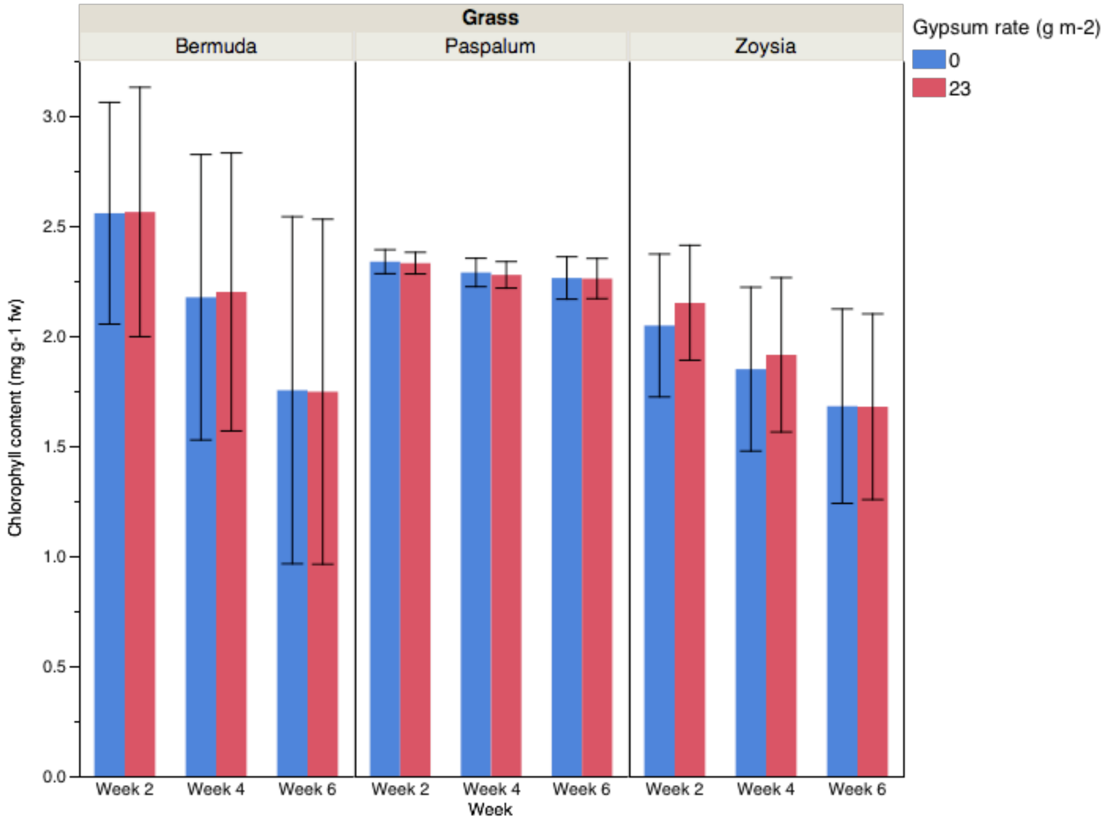


Figure 3.9. Effect of gypsum on chlorophyll content of three warm-season turfgrasses over the course of 6 weeks. Means were separated at P≤0.05 by protected LSD.

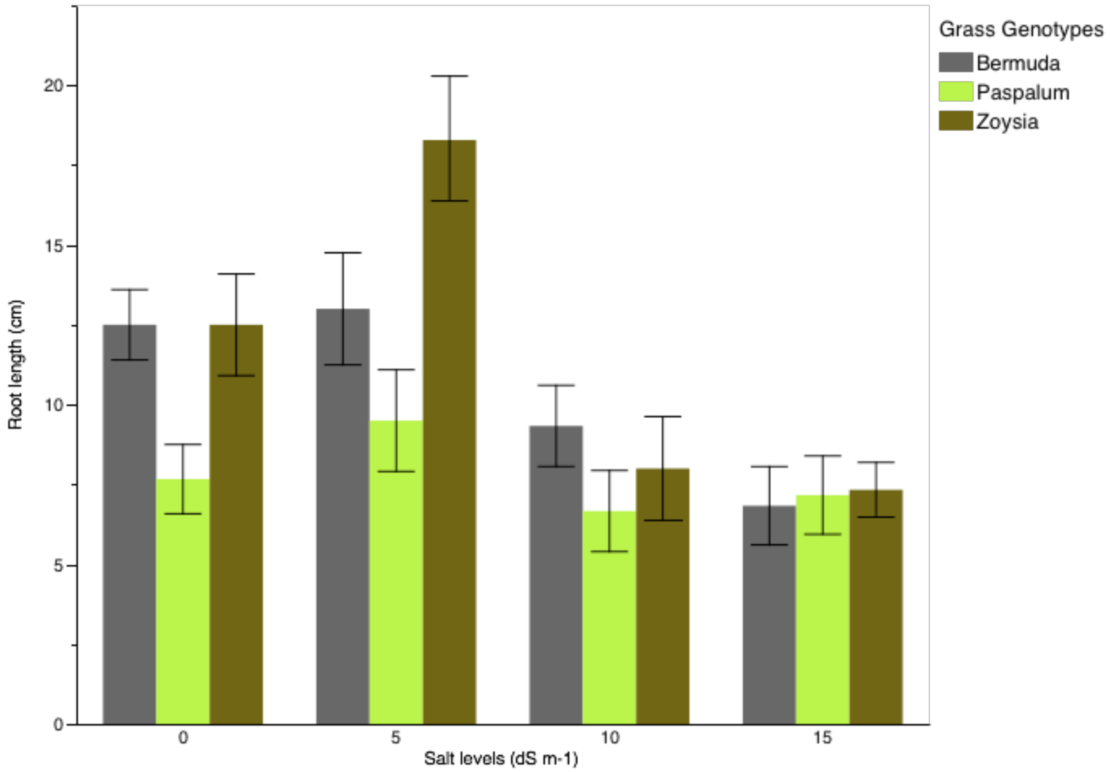


Figure 3.10. Root length of three warm-season turfgrasses as influenced by different salt regimes. Means were separated at P≤0.05 by protected LSD.

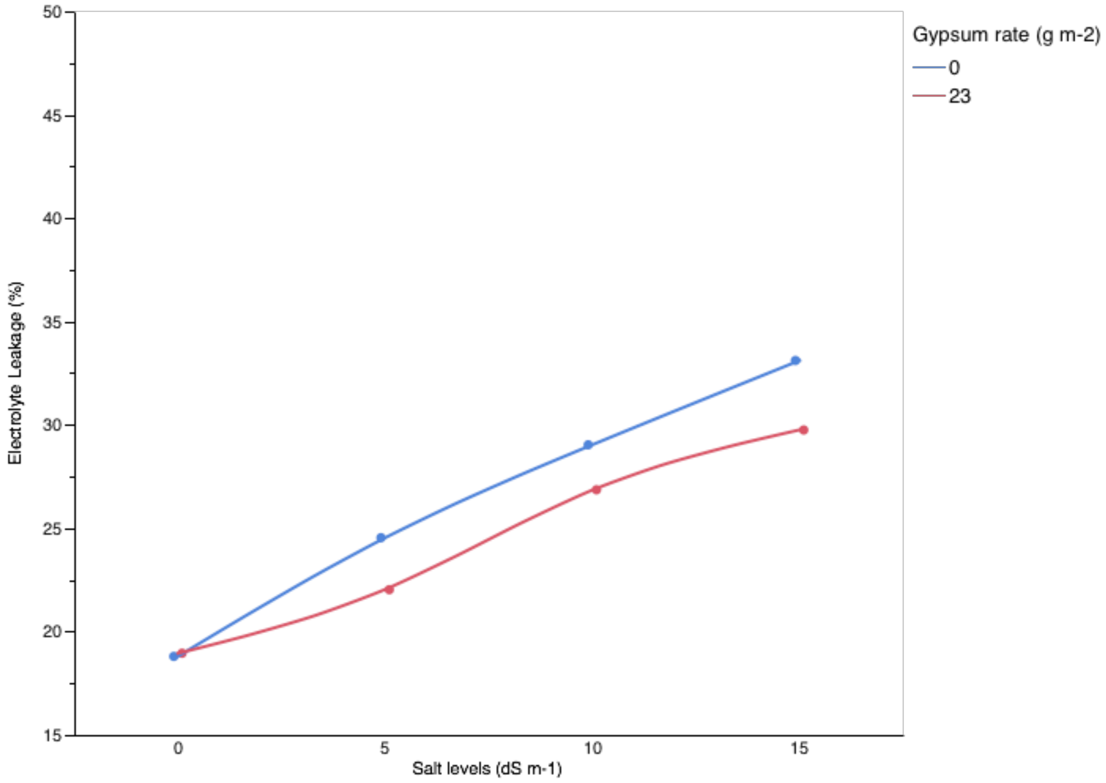


Figure 3.11. Effect of gypsum on electrolyte leakage of turfgrasses as influenced by different salinity regimes.

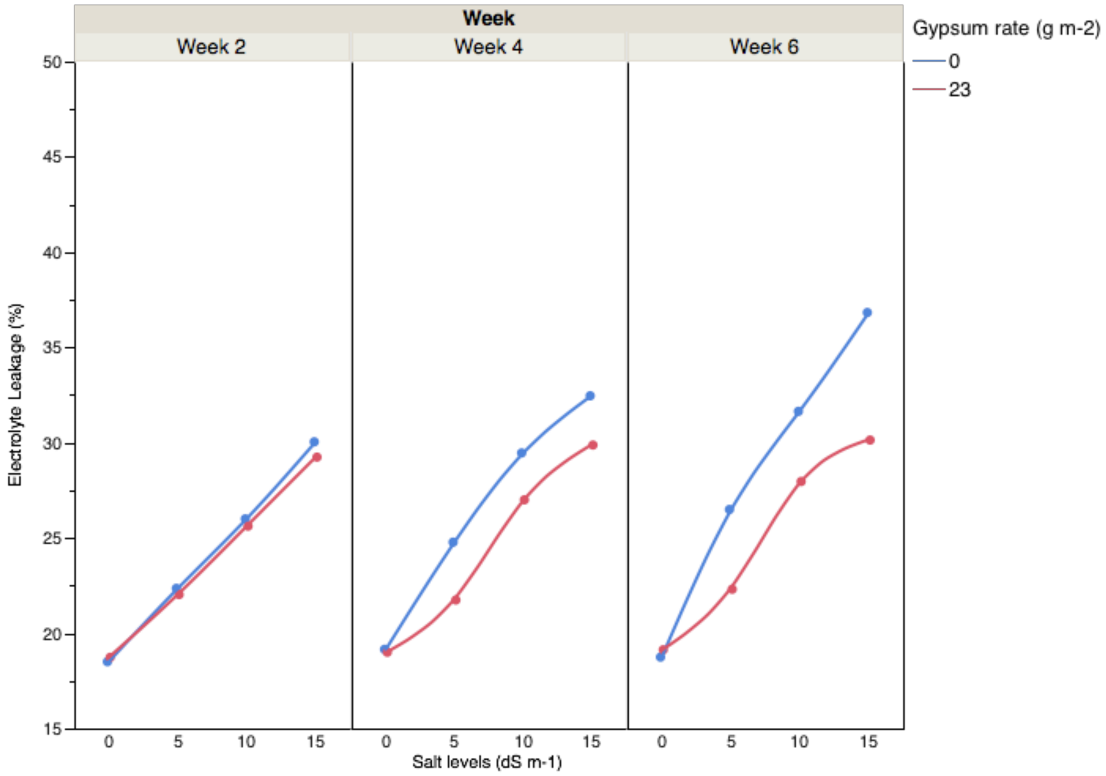


Figure 3.12. Effect of gypsum on electrolyte leakage of turfgrasses as influenced by different salinity regimes over the course of 6 weeks.

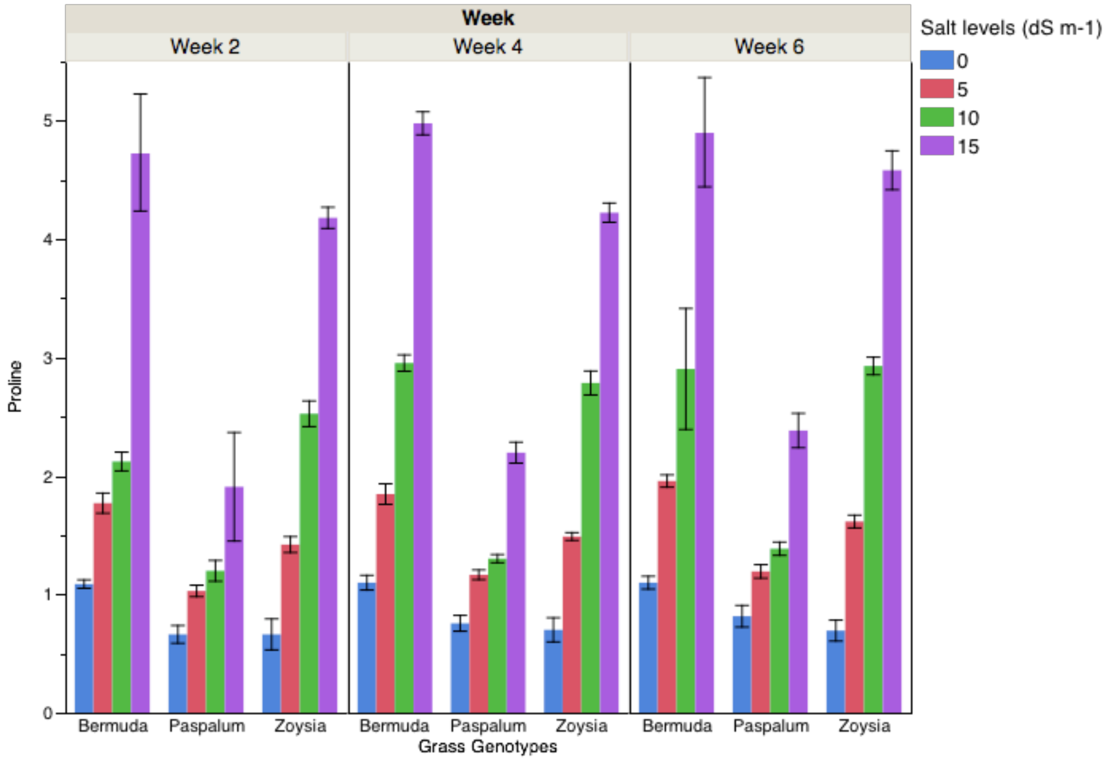


Figure 3.13. Proline content of three warm-season turfgrasses as influenced by salinity over the course of 6 weeks. Means were separated at P≤0.05 by protected LSD.

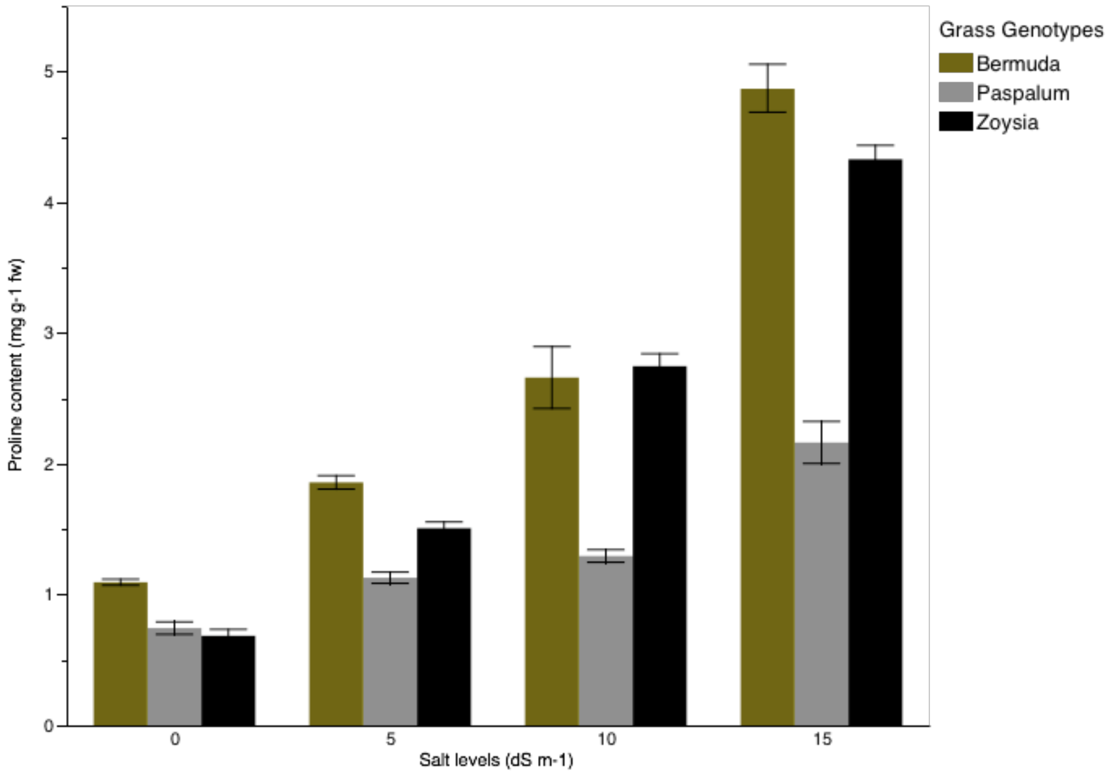


Figure 3.14. Effect of salinity on proline content in leaves of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

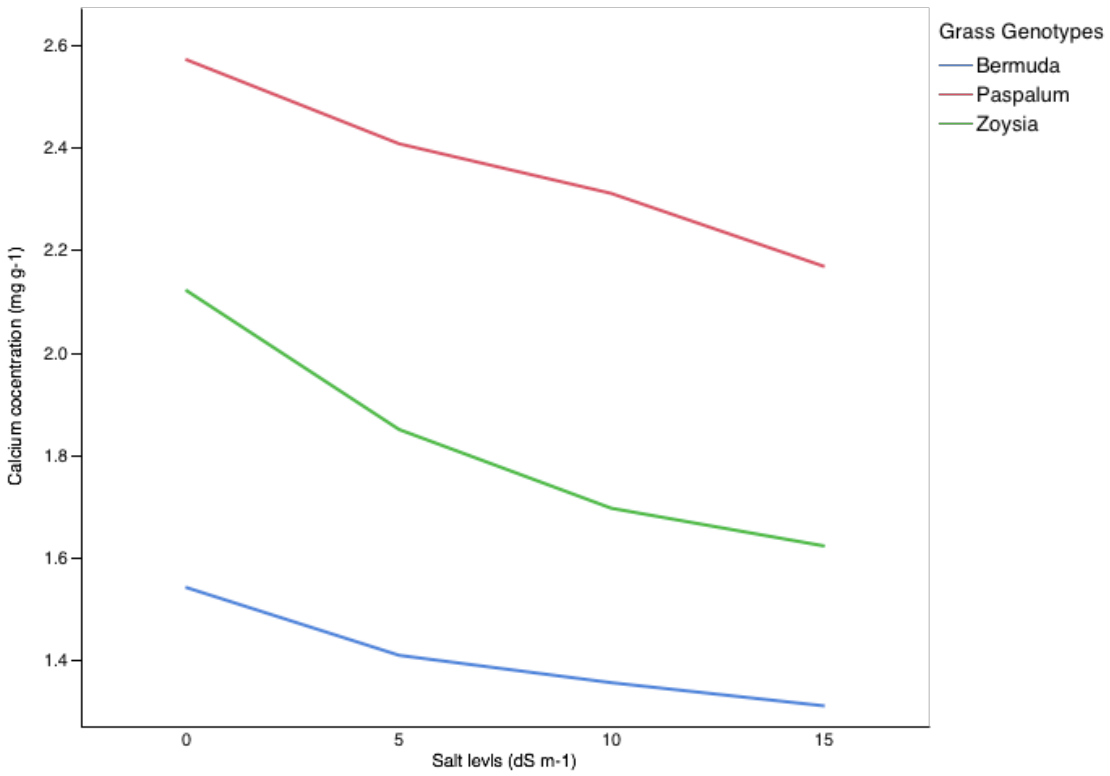


Figure 3.15. Calcium content in shoots of three warm-season turfgrasses as influenced by different salt regimes

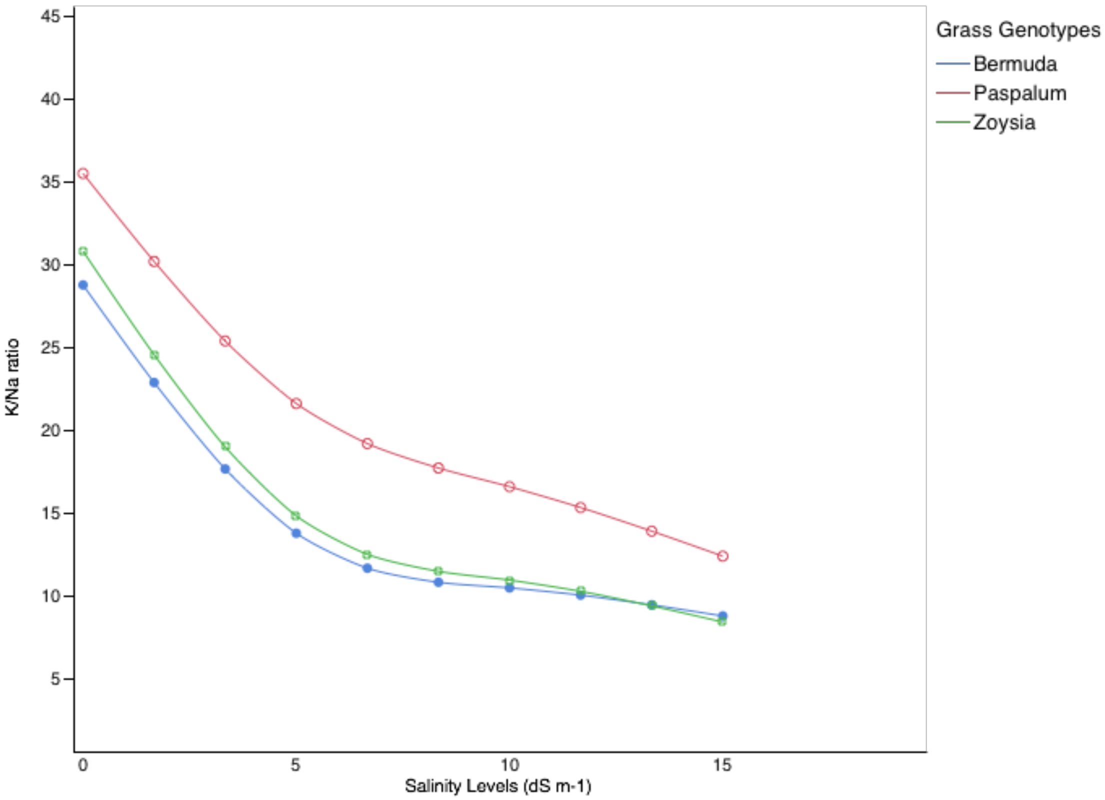


Figure 3.16. Relationship between salinity levels, grass species, and K/Na ratio in shoot.

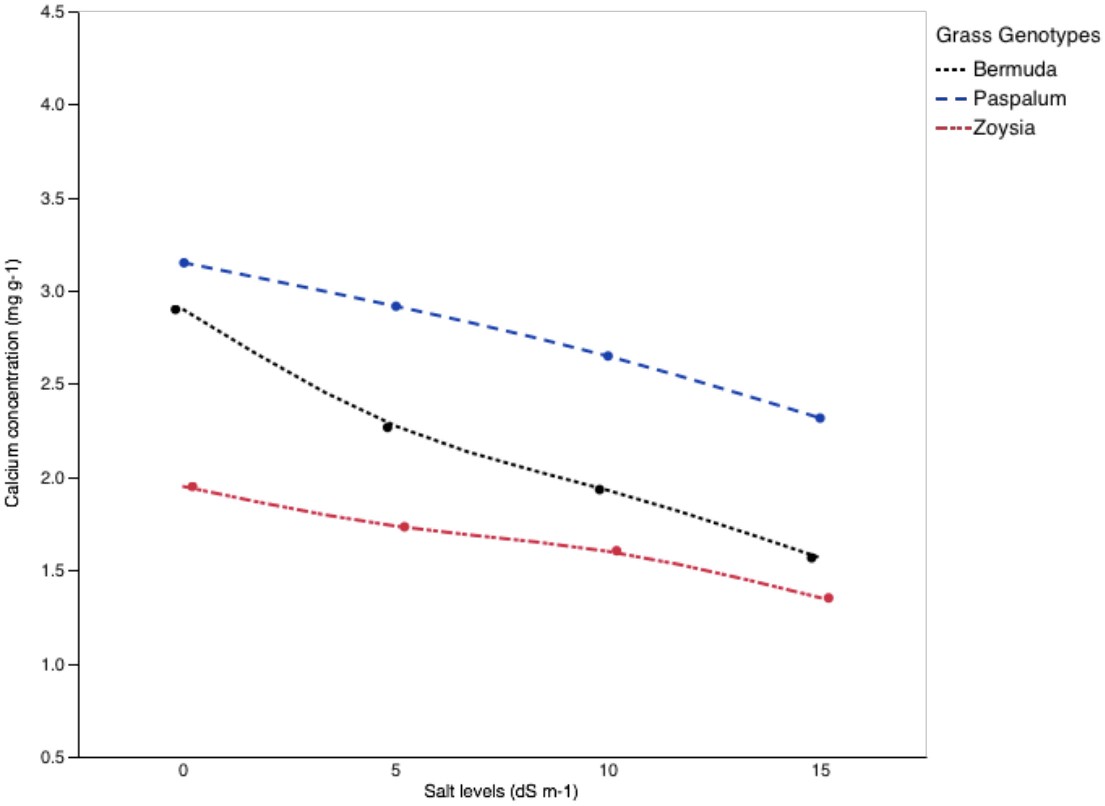


Figure 3.17. Calcium content in roots of three warm-season turfgrasses as influenced by different salt regimes

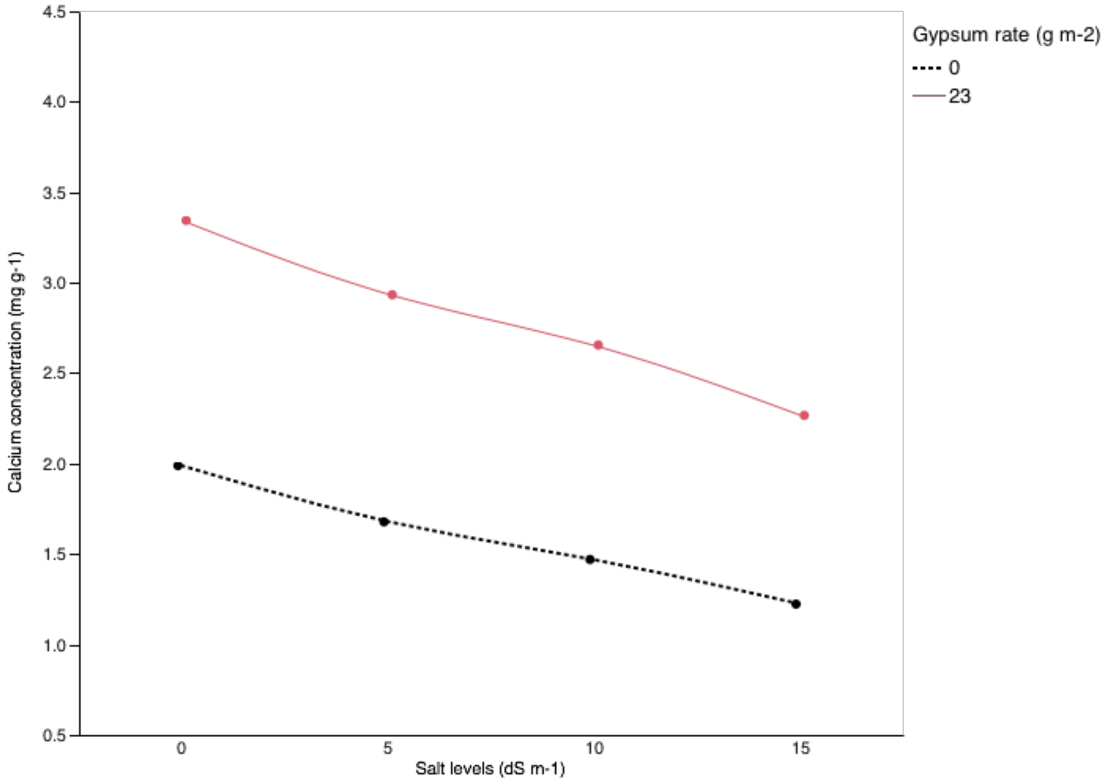


Figure 3.18. Effect of gypsum on calcium concentration in roots of three warm-season turfgrasses as influenced by different salinity regimes.

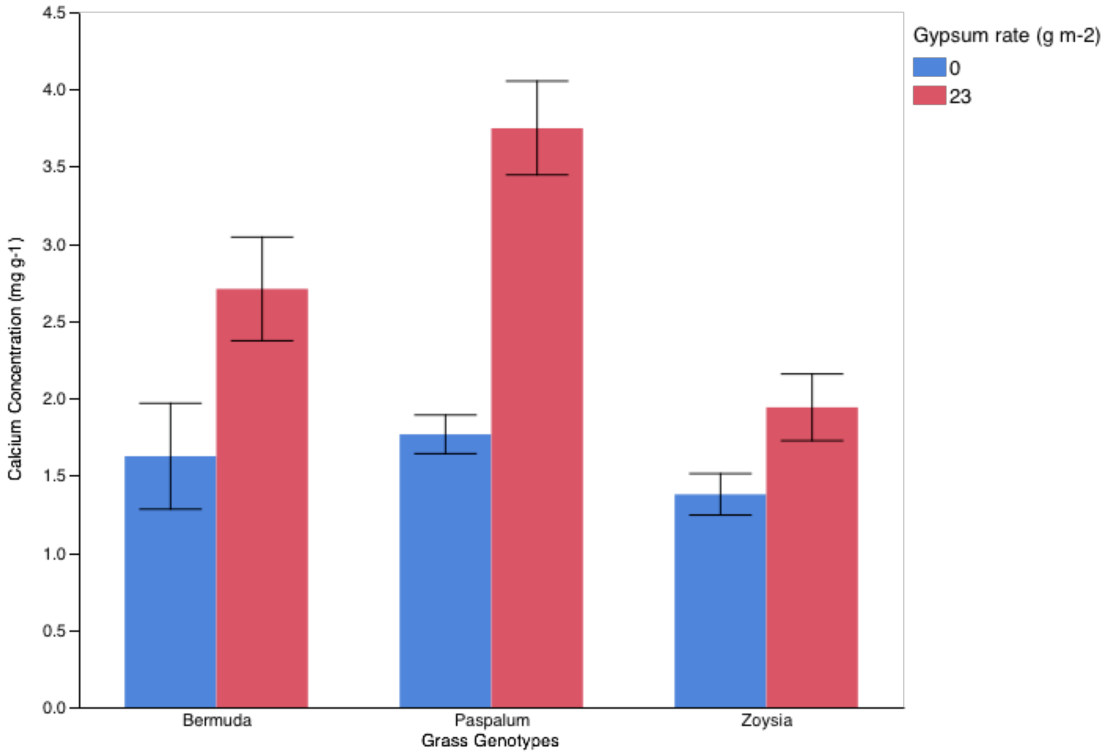


Figure 3.19. Effect of gypsum on calcium content in roots of three warm-season turfgrass. Means were separated at P≤0.05 by protected LSD.

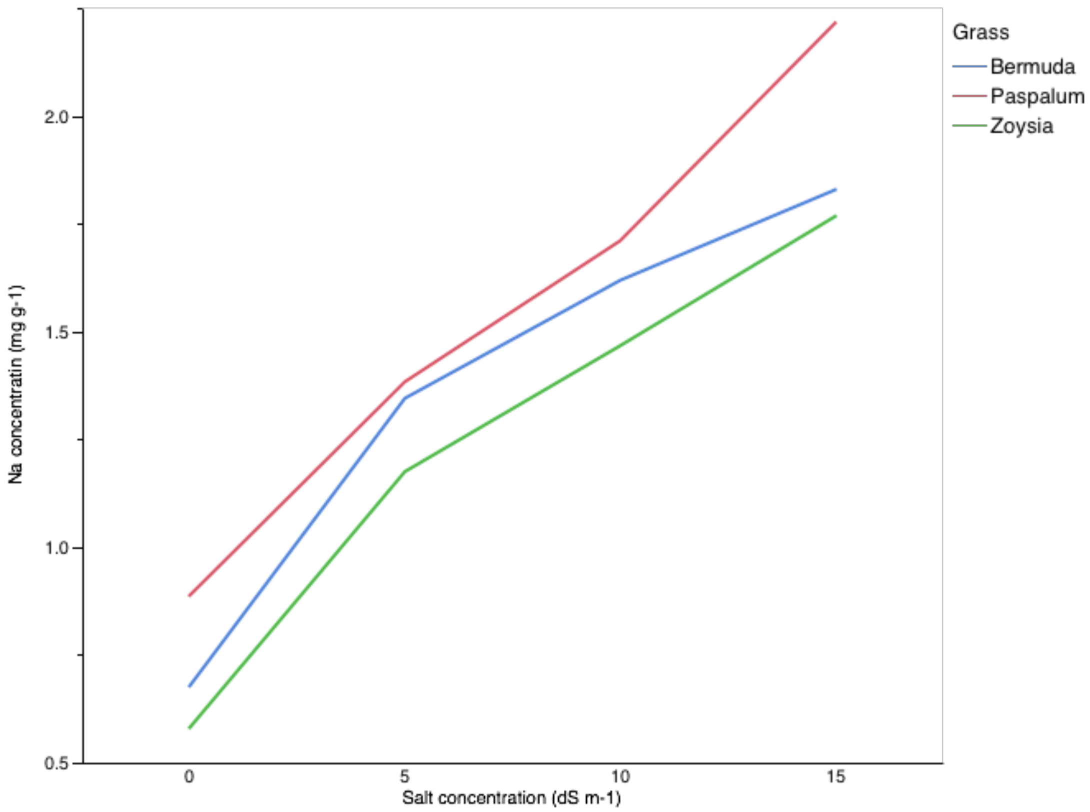


Figure 3.20. Effect of salinity on Sodium (Na) content in leaf tissue of three warm season turfgrasses.

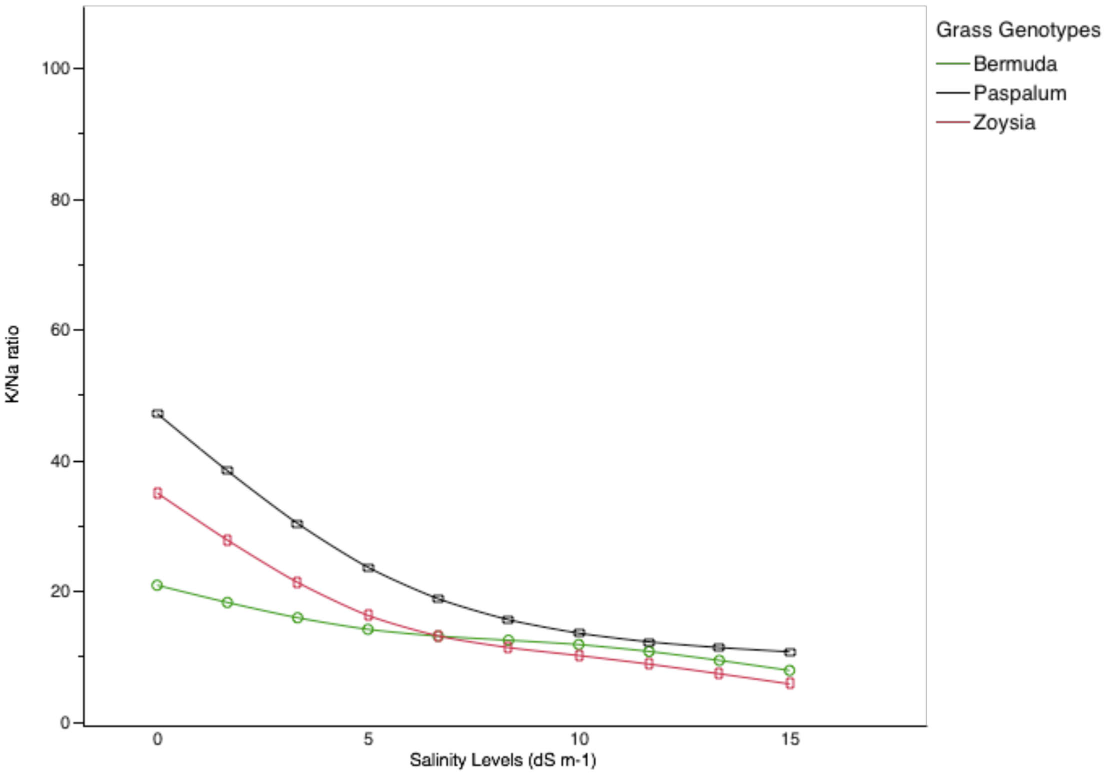


Figure 3.21. Relationship between salinity levels, grass species, and K/Na ratio in root.

Chapter 4: Summary and Future Research

Primary goal of this thesis were to determine the effect of additional calcium through gypsum application on warm-season turfgrass under salinity stress, grown in a non-calcareous sand based media. Secondary objective included determining the effect of Sodium (Na) and additional Calcium (Ca) on other cations in the leaf and root tissue of the turfgrass. The work of this thesis studied various morphological, physical and bio-chemical changes related to salinity stress and mitigation through calcium. In addition, a greenhouse study was conducted in 2013 to study the water loss during salt stress. While these results have answered many problems, they have also opened up new potential areas of research to be done in future.

Field evaluations of both salinity regime and gypsum regime produced interesting results. Irrigation water with salt (NaCl) induced salinity stress two weeks at the higher salt concentrations. After 6 weeks on continuous salinity treatment, gypsum application was done every two weeks at 23 g m^{-2}. Turf quality decreased following application of saline water and it continued even after gypsum application. Leaf firing increased with increase in salinity and additional gypsum did not have any impact. Osmotic stress due to high salt concentrations reduced the relative water content in the leaf and degrades the chlorophyll content. Gypsum treatment had minimal influence on any of these parameters.

Turfgrass clipping yield reduced with increased saline conditions and it did not stop following gypsum application. Proline content too increased with increase in salinity levels. Electrolyte leakage reduced when treated with supplemental calcium. Calcium is known to enhance the membrane stability and protect membrane from degradation. A comprehensive investigation of additional Ca in turfgrasses under salinity stress is needed to study the electrolyte leakage and membrane stability.

Additional calcium applied along with saline water on turfgrasses significantly affected the ion accumulation in shoot and root tissue. Increased Na concentrations in leaf reduced K and Mg in the leaf tissue as well as root tissue. Na damages the cell wall resulting of loss of K from the cells. The Ca in roots was significant affected by additional gypsum application. Findings from this study suggest that seashore paspalum is more salt tolerant compared to bermudagrass and zoysiagrass.

A greenhouse experiment conducted to examine the water loss or evapotranspiration suggested that at higher salinity levels water uptake by plants is less and ET is also small compared to the control salinity levels. Increase in gypsum decreased the ET in the grass genotypes.

Many questions regarding the effect of calcium supplementation on turfgrass under salinity stress is unanswered. Future studies involving long term evaluation is needed to fully understand the use of additional calcium. As our understanding of salinity stress mitigated with additional calcium by the turf industry should only help in decrease in electrolyte leakage not the salinity by itself. Thus, the use of calcium should be monitored on sand based medium since, no profound importance is noticed in turfgrass systems.

APPENDICES

Appendix-A: Tables

A-1. Physical and chemical properties of soil media used in the study at Tamil Nadu Agricultural University

S.NO	PARAMETERS	VALUE
1	Organic carbon	0.56%
2	pH	8.61
3	EC	0.06 dS m^{-1}
4	Available N	76 kg ha^{-1}
5	Available P(Olsen's)	9.9 kg ha^{-1}
6	Available K	204 kg ha^{-1}
7	Available Zn	0.72 ppm
8	Available Cu	1.79 ppm
9	Available Fe	0.54 ppm
10	Available Mn	2.41 ppm
11	Available S	28 ppm
12	Extractable Mg	69.5 ppm
13	Extractable Ca	115.9 ppm
14	Particle density	2.44 g cm-3
15	Bulk Density	1.41 g cm-3
16	Infiltration Rate	21 cm h-1
17	Total Porosity	49.1%

A-2. Sand size distribution analysis of the sand mix used in the study at Tamil Nadu Agricultural University.

Soil Separation (%)			**Sieve Size Fraction Retained (%)**					
Sand	Slit	Clay	No. 10 Gravel 2mm	No. 18 V. Coarse 1mm	No. 35 Coarse 0.5 mm	No. 60 Medium 0.25 mm	No. 100 Fine 0.15 mm	No. 270 V. Fine 0.05 mm
95.0	2.6	2.4	0.4	2.9	26.0	48.6	13	4.1

A-3. Weather Data during turfgrass establishment and treatment period at Tamil Nadu Agricultural University, Coimbatore.

Month (2014)	**Temperature (^{o}C)**		**Relative humidity**		**Rainfall (mm)**
	Maximum	**Minimum**	**at 07.00 hrs**	**at 14.00 hrs**	
January	30.10	19.70	84.00	41.97	0.0
February	32.40	24.40	79.00	39.39	0.0
March	34.50	22.30	74.00	34.48	0.0
April	36.60	24.30	81.00	39.17	0.0
May	34.30	24.40	84.77	52.29	125.8
June	32.84	24.32	78.57	52.73	10.6
July	30.67	23.29	77.61	59.77	41.20
August	30.55	23.10	81.88	59.29	2.38
September	31.82	22.66	85.4	55.03	3.01
October	30.06	22.53	93.03	68	11.35

Table A-4. Turf quality as influenced by salinity regime, gypsum regime, and species for 6 weeks under open field conditions.

Main effects	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
-----------------------------------(1-9, >6)-----------------------------------						
Salinity (S) (dS/m)						
Control	8.47	8.45	8.39	8.25	8.30	8.34
5	7.62	6.94	6.84	6.47	6.19	6.03
10	6.95	6.46	6.14	5.91	5.55	5.40
15	6.62	5.85	5.23	5.11	5.02	4.62
Gypsum Regime (C) (g/m^2)						
Control	8.47	8.45	8.39	8.25	8.30	8.34
23	8.52	8.41	8.38	8.26	8.15	8.22
Genotype (G)						
Zoysia	8.54	8.31	8.41	8.19	8.22	8.26
Bermuda	8.12	8.00	7.66	7.60	7.63	7.68
Paspalum	8.76	9.03	9.10	8.96	9.05	9.08
ANOVA						
Grass	NS	NS	*	*	NS	NS
Salt	***	***	***	***	***	***
Gypsum	NS	NS	NS	NS	NS	NS
Grass*Salt	**	**	***	***	***	***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-5. Leaf firing as influenced by salinity regime, gypsum regime, and species for 6 weeks under open field conditions.

Main effects	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
------------------------------------(%)------------------------------------						
Salinity (S) (dS/m)						
Control	0.05	0.24	-0.16	-0.02	0.03	-0.12
5	9.69	14.22	18.40	20.09	22.23	23.80
10	16.69	21.94	25.65	28.48	32.45	33.85
15	26.52	32.44	36.40	37.73	40.45	41.52
Gypsum Regime (C) (g/m^2)						
Control	0.05	0.24	-0.16	-0.02	0.03	-0.12
23	-0.05	-0.24	0.16	0.02	-0.03	0.12
Genotype (G)						
Zoysia	0.11	2.74	3.36	3.93	4.40	4.17
Bermuda	6.94	7.11	8.24	7.97	9.07	8.71
Paspalum	-6.91	-9.12	-12.08	-11.97	-13.37	-13.23
ANOVA						
Grass	NS	NS	NS	NS	NS	NS
Salt	***	***	***	***	***	***
Gypsum	NS	NS	NS	NS	NS	NS
Grass*Salt	***	***	***	***	***	***
Salt*Gypsum	NS	NS	NS	*	NS	NS

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level

NS Not significant at any level

† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-6. Relative water content as influenced by salinity regime, gypsum regime, and species for 6 weeks under open field conditions.

Main effects	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
----------------------------------(%)----------------------------------						
Salinity (S) (dS/m)						
Control	82.95	83.41	83.44	83.72	83.94	83.16
5	73.82	70.57	67.75	64.47	61.68	59.51
10	67.17	63.18	59.81	58.11	54.92	52.63
15	62.36	56.24	52.62	48.70	47.32	44.82
Gypsum Regime (C) (g/m^2)						
Control	82.95	83.41	83.44	83.72	83.94	83.16
23	83.62	83.44	83.93	83.59	83.98	83.60
Genotype (G)						
Zoysia	79.69	80.15	79.51	79.06	80.40	80.25
Bermuda	77.08	76.19	75.58	75.21	75.41	73.41
Paspalum	92.09	93.89	95.24	96.89	96.00	95.80
ANOVA						
Grass	***	**	**	**	**	**
Salt	***	***	***	***	***	***
Gypsum	NS	NS	NS	NS	NS	NS
Grass*Salt	***	***	***	***	***	***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
NS Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-7. Clipping weight as influenced by salinity regime, gypsum regime, and species for 6 weeks under open field conditions.

Main effects	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
-----------------------------------(g m^{-2})-----------------------------------						
Salinity (S) (dS/m)						
Control	2.00	1.99	2.01	1.99	2.00	1.99
5	1.95	1.93	1.93	1.88	1.85	1.80
10	1.87	1.80	1.70	1.63	1.53	1.46
15	1.75	1.65	1.58	1.46	1.26	1.15
Gypsum Regime (C) (g/m^2)						
Control	2.00	1.99	2.01	1.99	2.00	1.99
23	1.99	1.99	2.00	2.00	2.01	1.98
Genotype (G)						
Zoysia	1.44	1.41	1.45	1.42	1.42	1.43
Bermuda	1.42	1.42	1.40	1.39	1.42	1.39
Paspalum	3.15	3.15	3.17	3.15	3.17	3.14
ANOVA						
Grass	***	***	***	***	***	***
Salt	***	***	***	***	***	***
Gypsum	NS	NS	NS	NS	NS	NS
Grass*Salt	***	***	**	**	NS	**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
NS Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-8. Electrolyte leakage content in leaf tissue of zoysiagrass, bermudagrass and seashore paspalum as influenced by salinity regime, gypsum regime, and grass genotypes at three harvest events (2, 4 and 6 weeks after initiation of gypsum treatment).

Main effects	**Week 2**	**Week 4**	**Week 6**
	---------------------------(%)---------------------------		
Salinity (S) (dS/m)			
Control	18.62	19.08	18.94
5	22.19	23.26	24.40
10	25.81	28.23	29.80
15	29.64	31.17	33.48
Gypsum Regime (C) (g/m^2)			
Control	24.23	26.46	28.43
23	23.90	24.40	24.88
Genotype (G)			
Zoysia	23.25	23.99	25.72
Bermuda	27.40	29.17	30.73
Paspalum	21.55	23.14	23.53
ANOVA			
Grass	NS	NS	NS
Salt	***	***	***
Grass*Salt	***	***	***
Salt*Gypsum	NS	**	***
Grass*Salt*Gypsum	NS	NS	**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-9. Proline content in leaf tissue of zoysiagrass, bermudagrass and seashore paspalum as influenced by salinity regime, gypsum regime, and grass genotypes at three harvest events (2, 4 and 6 weeks after initiation of gypsum treatment).

Main effects	**Week 2**	**Week 4**	**Week 6**
	-----------------------(mg g^{-1} fw)----------------------		
Salinity (S) (dS/m)			
Control	0.80	0.85	0.87
5	1.41	1.50	1.59
10	1.95	2.35	2.41
15	3.61	3.80	3.96
Gypsum Regime (C) (g/m^2)			
Control	1.97	2.11	2.21
23	1.92	2.14	2.20
Genotype (G)			
Zoysia	2.20	2.30	2.45
Bermuda	2.43	2.72	2.72
Paspalum	1.20	1.35	1.44
ANOVA			
Grass	**	***	*
Salt	***	***	***
Grass*Salt	***	***	***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-10. Chlorophyll content in leaf tissue of zoysiagrass, bermudagrass and seashore paspalum as influenced by salinity regime, gypsum regime, and grass genotypes at three harvest events (2, 4 and 6 weeks after initiation of gypsum treatment).

Main effects	**Week 2**	**Week 4**	**Week 6**
	-----------------------(mg g^{-1} fw)----------------------		
Salinity (S) (dS/m)			
Control	3.00	2.98	3.00
5	2.37	2.05	1.69
10	2.10	1.84	1.54
15	1.86	1.60	1.36
Gypsum Regime (C) (g/m^2)			
Control	2.31	2.10	1.90
23	2.35	2.13	1.90
Genotype (G)			
Zoysia	2.10	1.88	1.68
Bermuda	2.56	2.19	1.75
Paspalum	2.33	2.28	2.26
ANOVA			
Grass	***	***	***
Salt	***	***	***
Grass*Salt	***	***	***

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
NS Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-11. Effect of salinity and gypsum regime on root length, root Calcium (Ca), Magnesium (Mg), Sodium (Na), and Potassium (k) concentrations of three turfgrass genotypes exposed to salinity at the conclusion of the study.

Main effects	**Root length**	**Ca**	**Mg**	**Na**	**K**
	-------(cm)-------	**----------------------($mg\ g^{-1}$)--------------------**			
Salinity (S) (dS/m)					
Control	10.33b	1.99	3.39	0.82	20.55
5	13.67a	1.68	3.01	1.20	20.33
10	7.56c	1.47	2.86	1.64	19.54
15	7.00c	1.22	2.51	2.20	16.23
Gypsum Regime (C) (g/m^2)					
Control	10.33a	1.99	3.39	0.82	20.55
23	11.44a	3.34	3.54	0.70	22.88
Genotype (G)					
Zoysia	11.54a	1.63	3.27	0.50	16.11
Bermuda	10.42a	2.33	2.97	1.23	17.25
Paspalum	7.75b	2.00	3.93	0.73	28.27
ANOVA					
Grass	***	***	***	**	***
Salt	***	***	***	***	***
Gypsum	NS	***	NS	NS	*
Grass*Salt	**	**	NS	**	NS
Grass*Gypsum	NS	***	NS	NS	NS
Salt*Gypsum	NS	*	NS	NS	NS

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
^{NS} Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

Table A-12. Effect of salinity and gypsum regime on shoot Calcium (Ca), Magnesium (Mg), Sodium (Na), and Potassium (k) concentrations of three turfgrass genotypes exposed to salinity at the conclusion of the study.

Main effects	Ca	Mg	Na	K
	---------------------------(mg g^{-1})-------------------------			
Salinity (S) (dS/m)				
Control	2.04	3.54	0.70	22.60
5	1.85	3.36	1.29	21.77
10	1.75	3.25	1.58	20.74
15	1.66	3.10	1.92	19.52
Gypsum Regime (C) (g/m^2)				
Control	2.04	3.54	0.73	22.60
23	2.12	3.57	0.70	22.21
Genotype (G)				
Zoysia	2.00	2.98	1.25	17.94
Bermuda	1.58	3.36	1.37	19.20
Paspalum	2.54	4.28	1.55	30.65
ANOVA				
Grass	***	***	*	***
Salt	***	***	***	***
Gypsum	**	NS	NS	NS
Grass*Salt	*	***	*	NS
Salt*Gypsum	NS	*	NS	NS
Grass*Salt*Gypsum	NS	NS	NS	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level
NS Not significant at any level
† Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

APPENDIX-B

Figure B-1.Turf quality of three warm-season turfgrasses as influenced by salinity and gypsum.

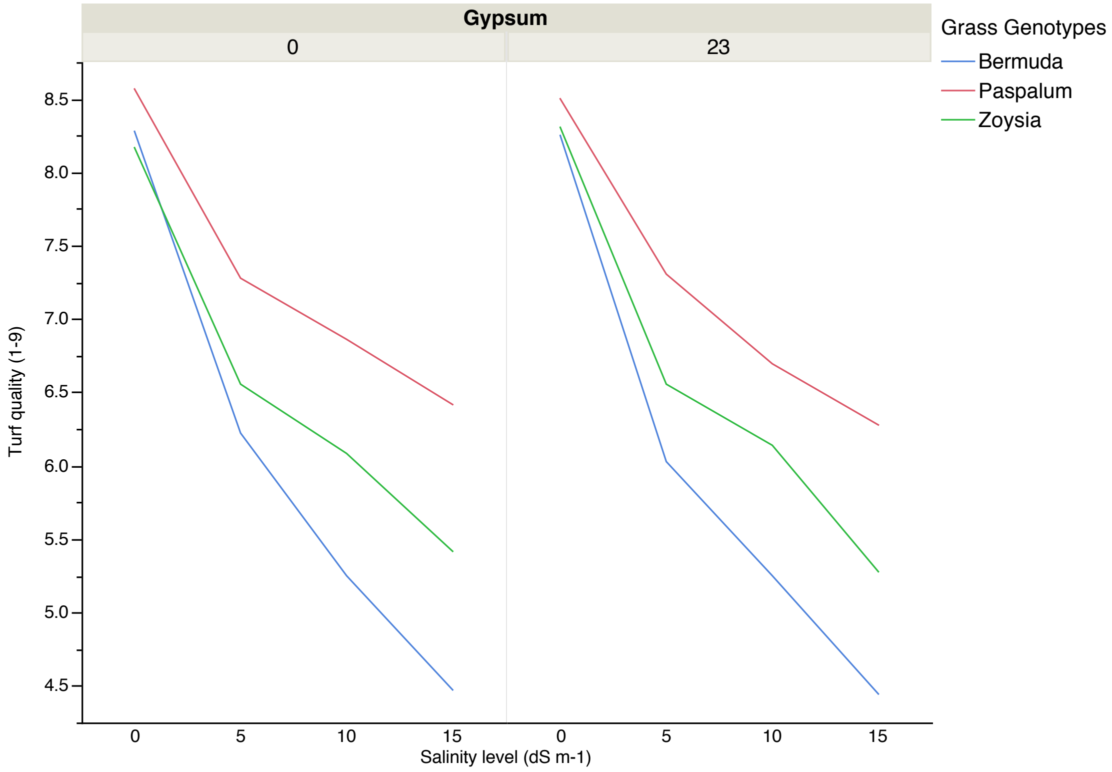


Figure B-2. Leaf firing of three warm-season turfgrasses as influenced by salinity and gypsum

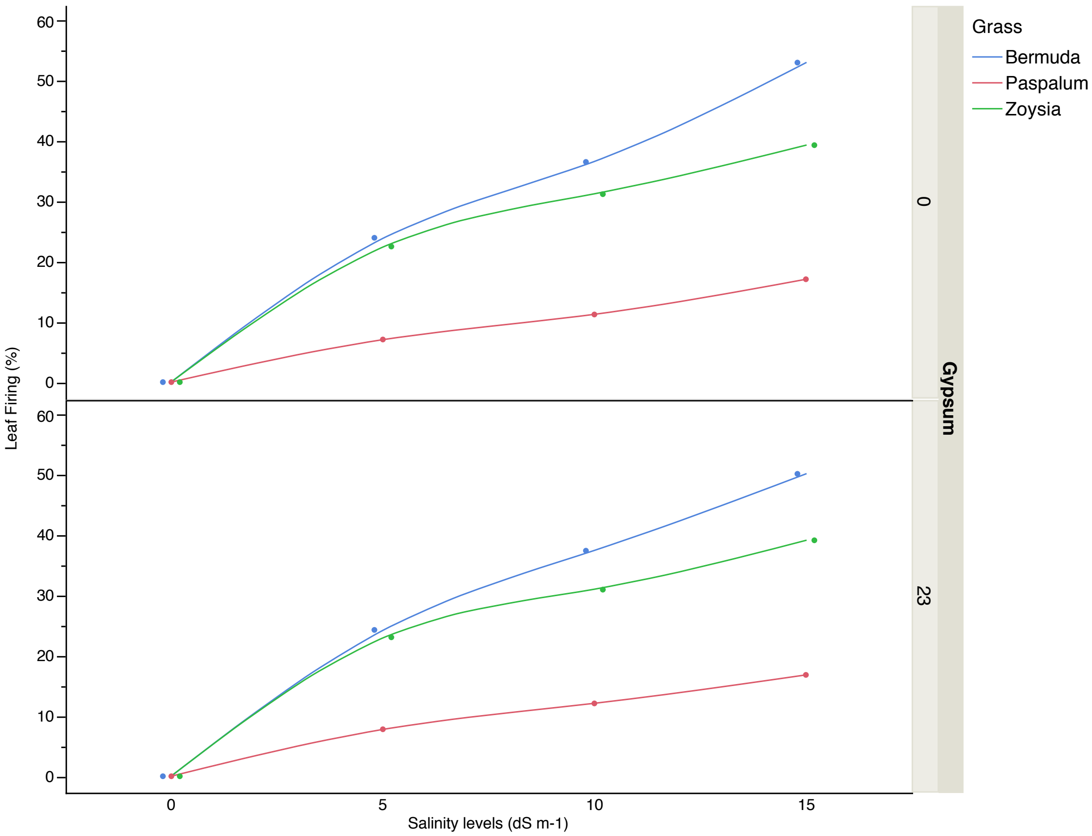


Figure B-3. The effect of turf quality on leaf firing of the warm season turfgrasses under salinity stress.

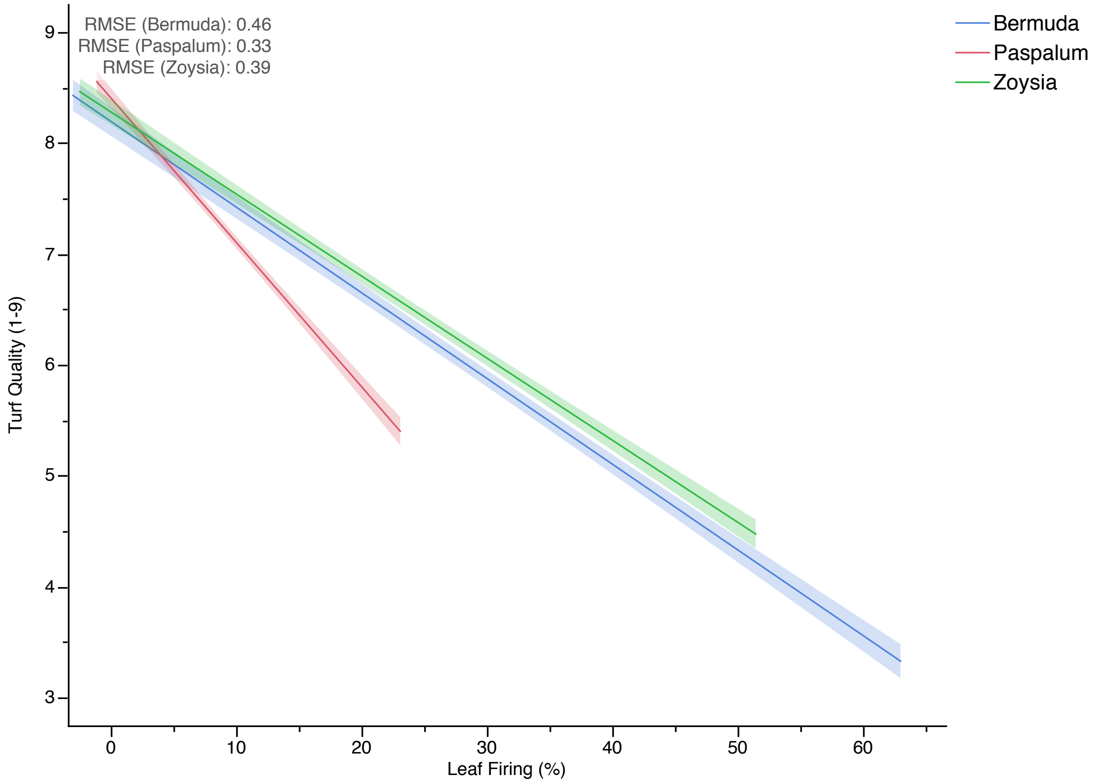


Figure B-4. The effect of salinity and gypsum on chlorophyll content in the turfgrass leaves over the course of study.

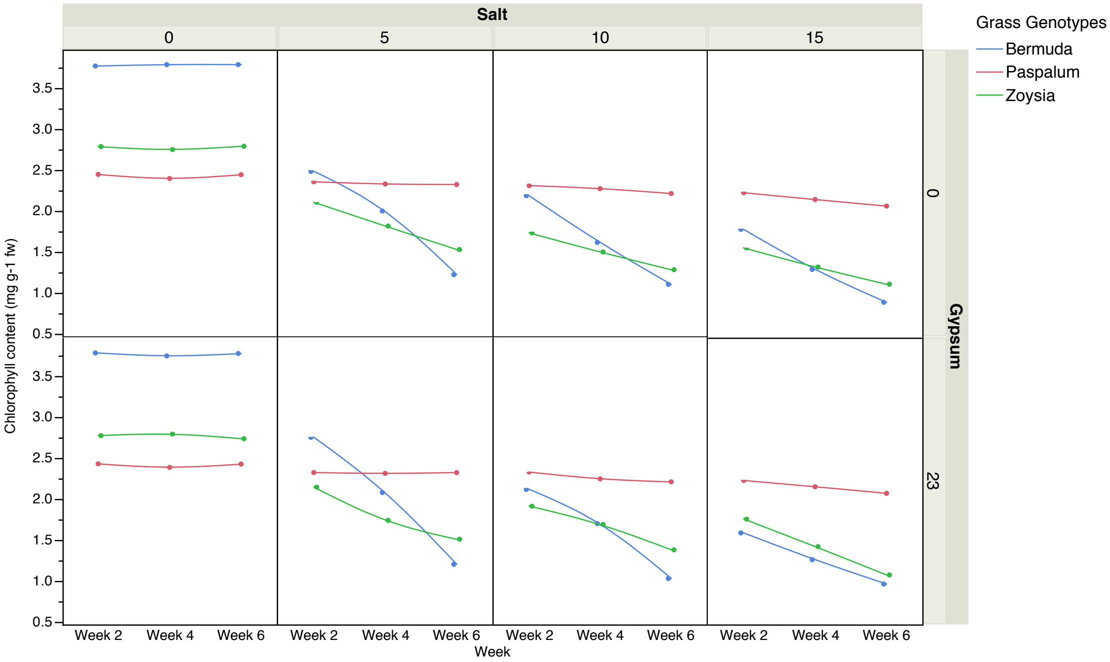


Figure B-5. Effect of salinity on Magnesium (Mg) content in leaf tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

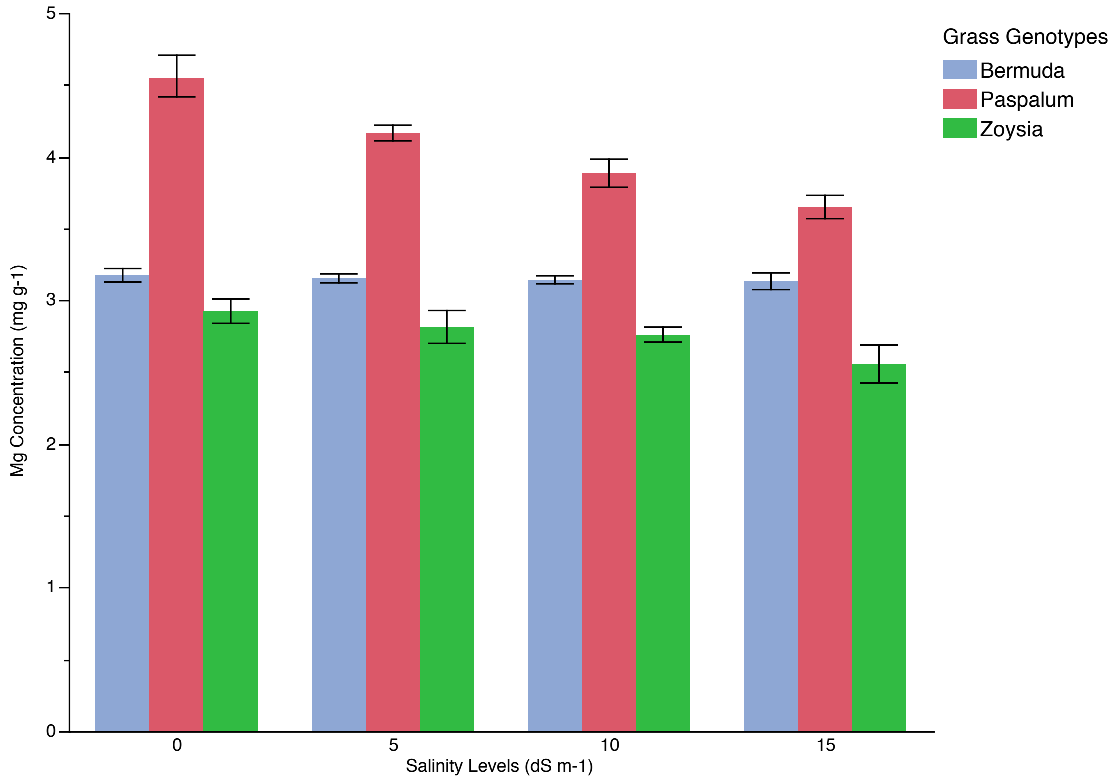


Figure B-6. Effect of salinity and gypsum on Potassium (K) content in leaf tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

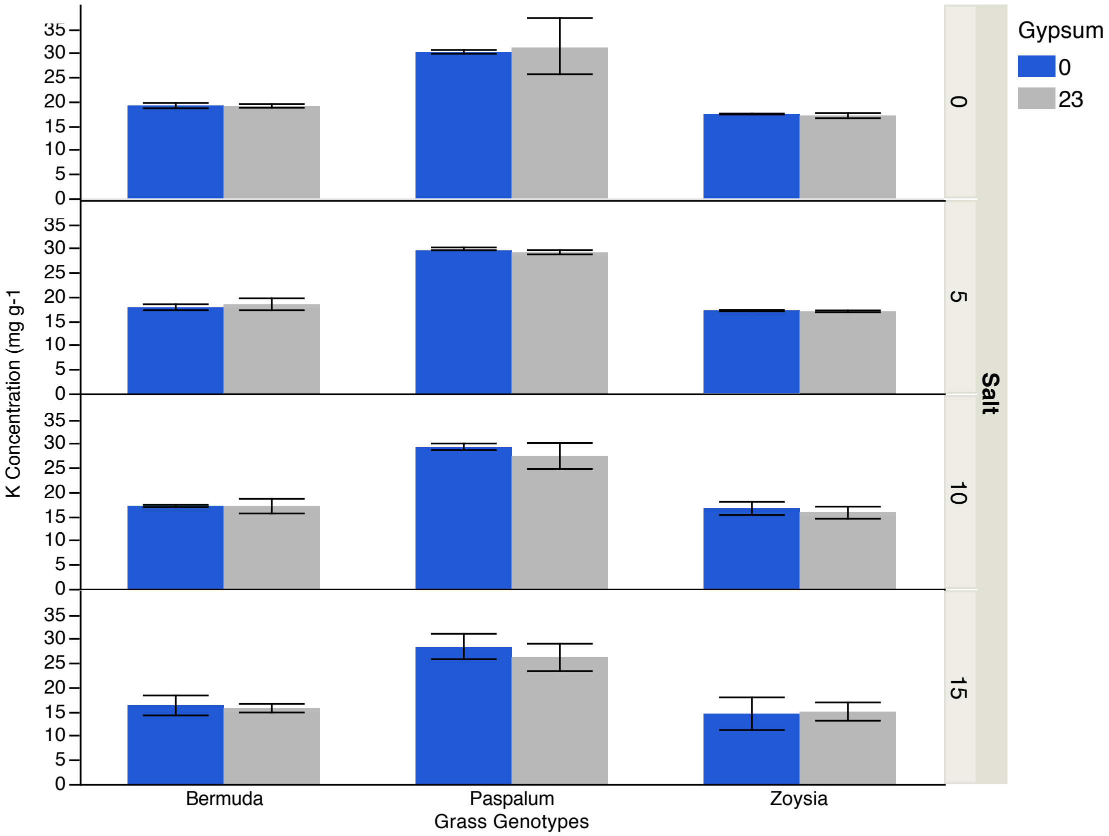


Figure B-7. Effect of salinity and gypsum on root length of the grass genotypes at the end of the study.

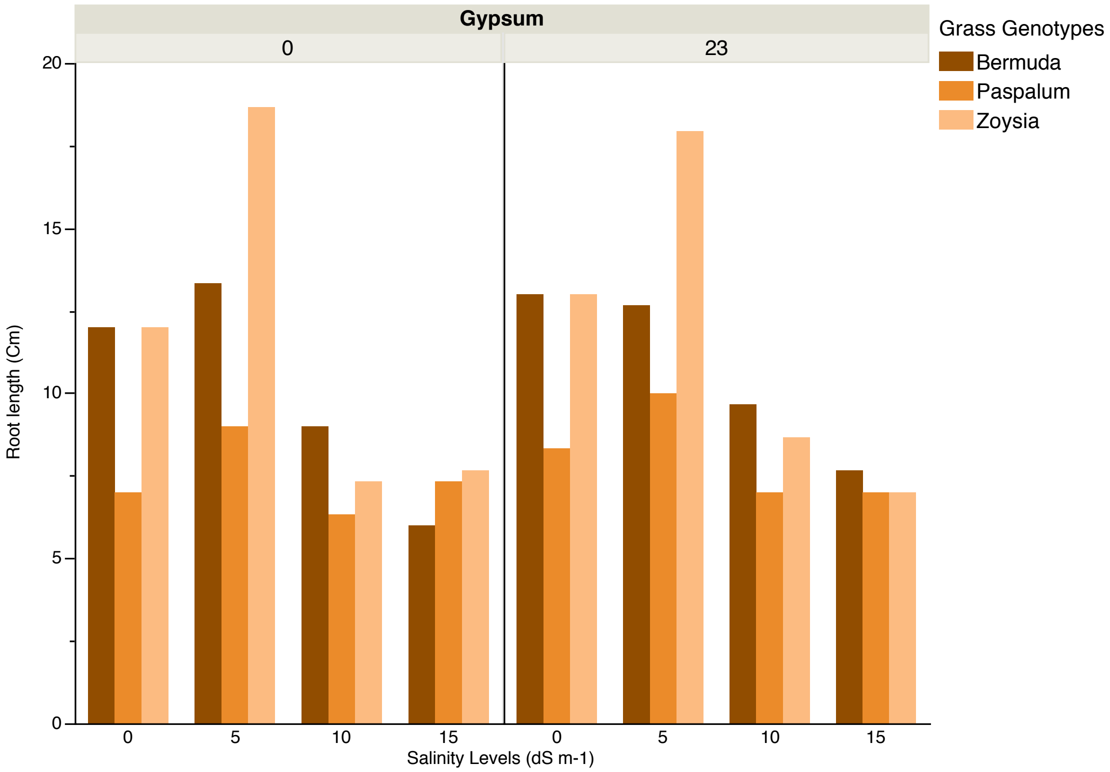


Figure B-8. Effect of salinity and gypsum on Magnesium (Mg) content in root tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

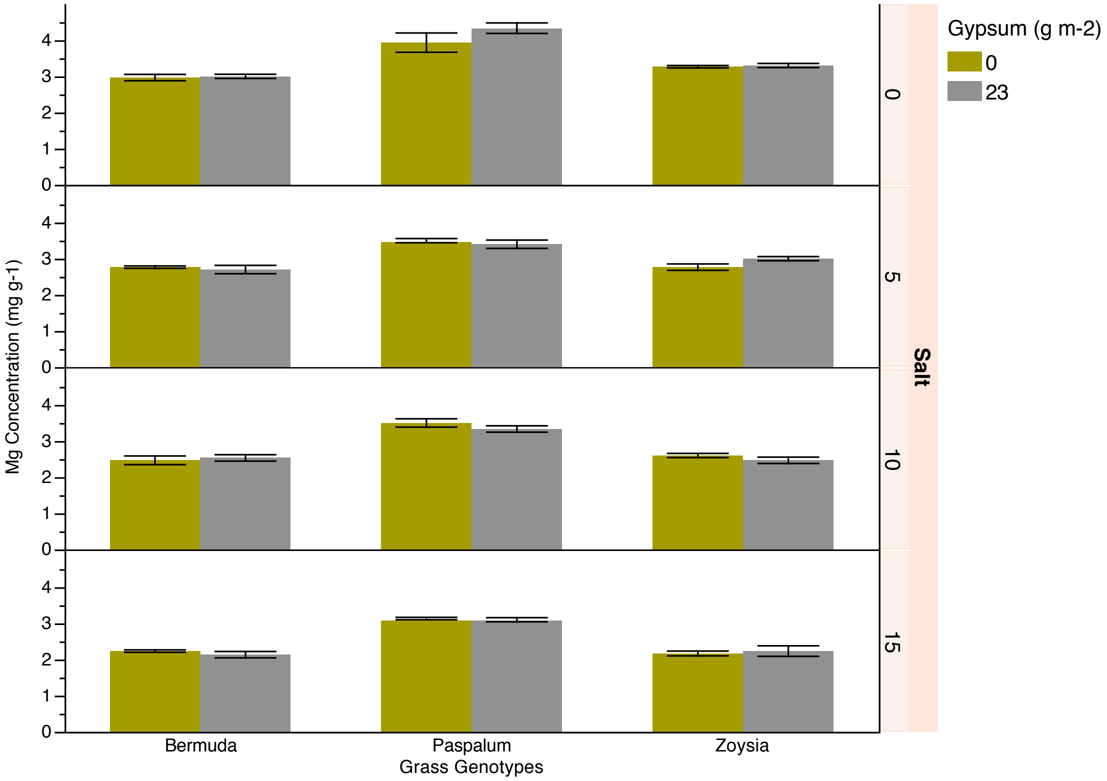


Figure B-9. Effect of salinity on Sodium (Na) content in root tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

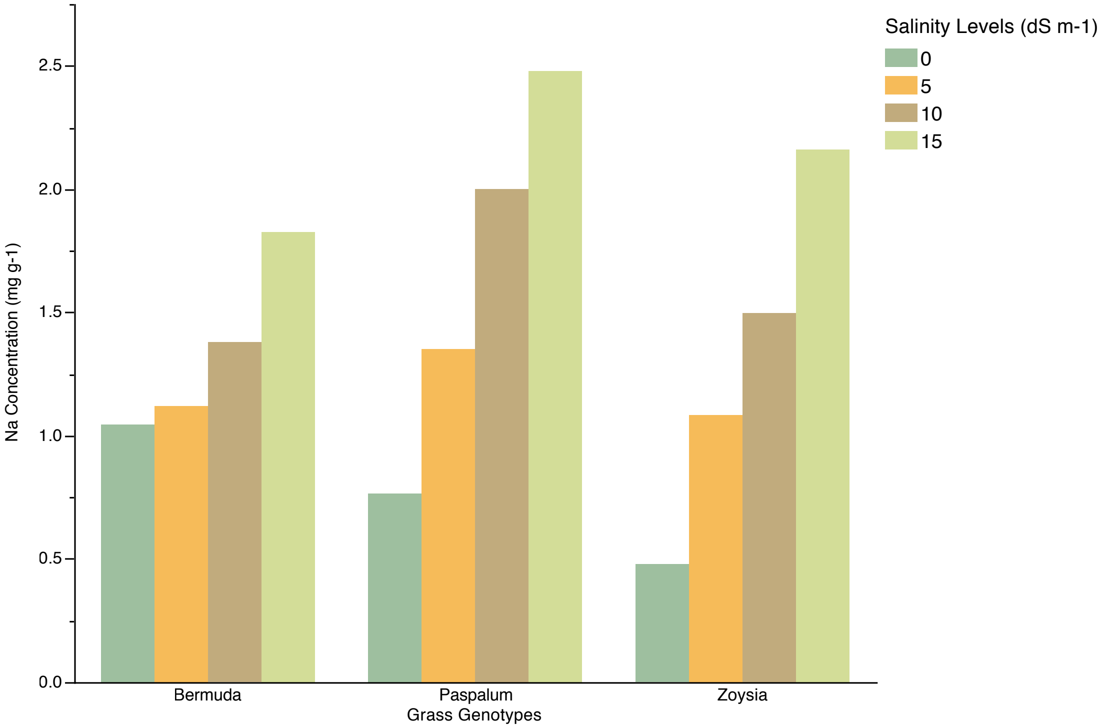


Figure B-10. Salinity and gypsum effect on electrolyte leakage of the turfgrasses.

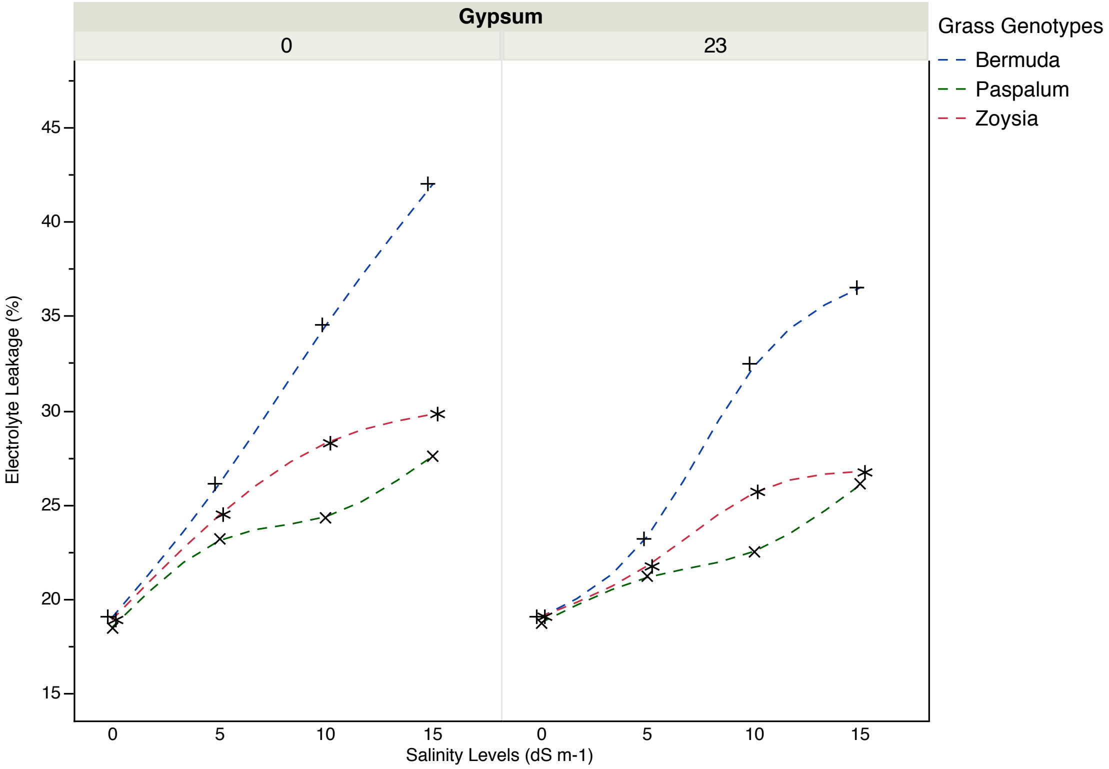


Figure B-11. Effect of salinity and gypsum on Calcium (Ca) content in leaf tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.

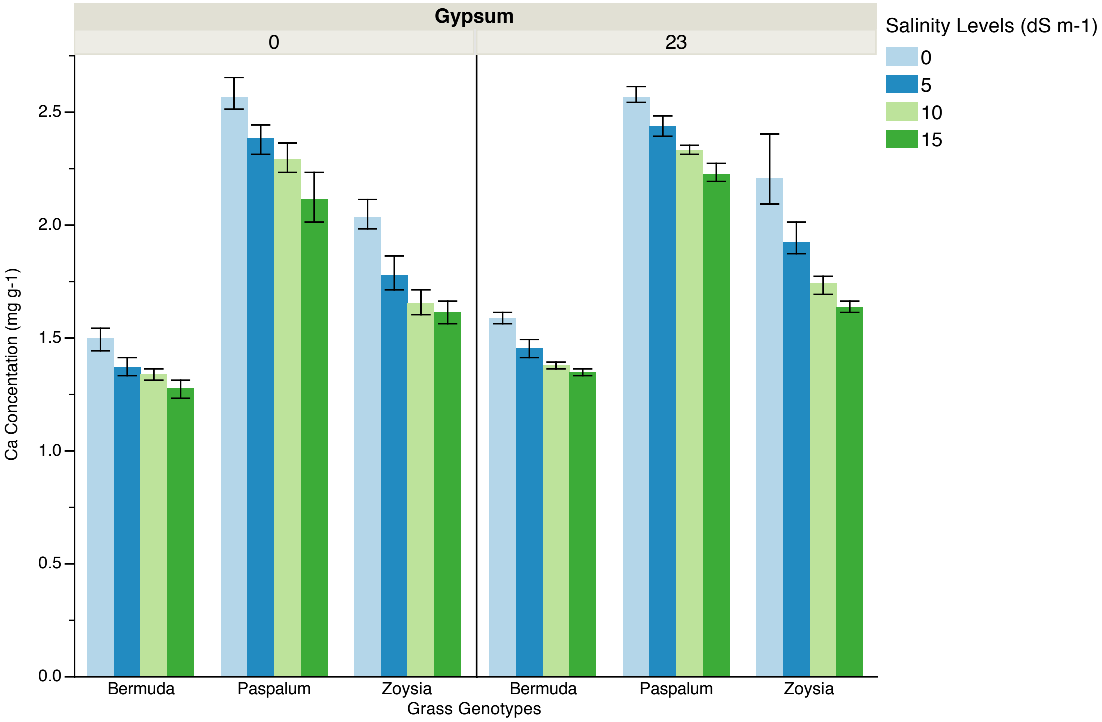
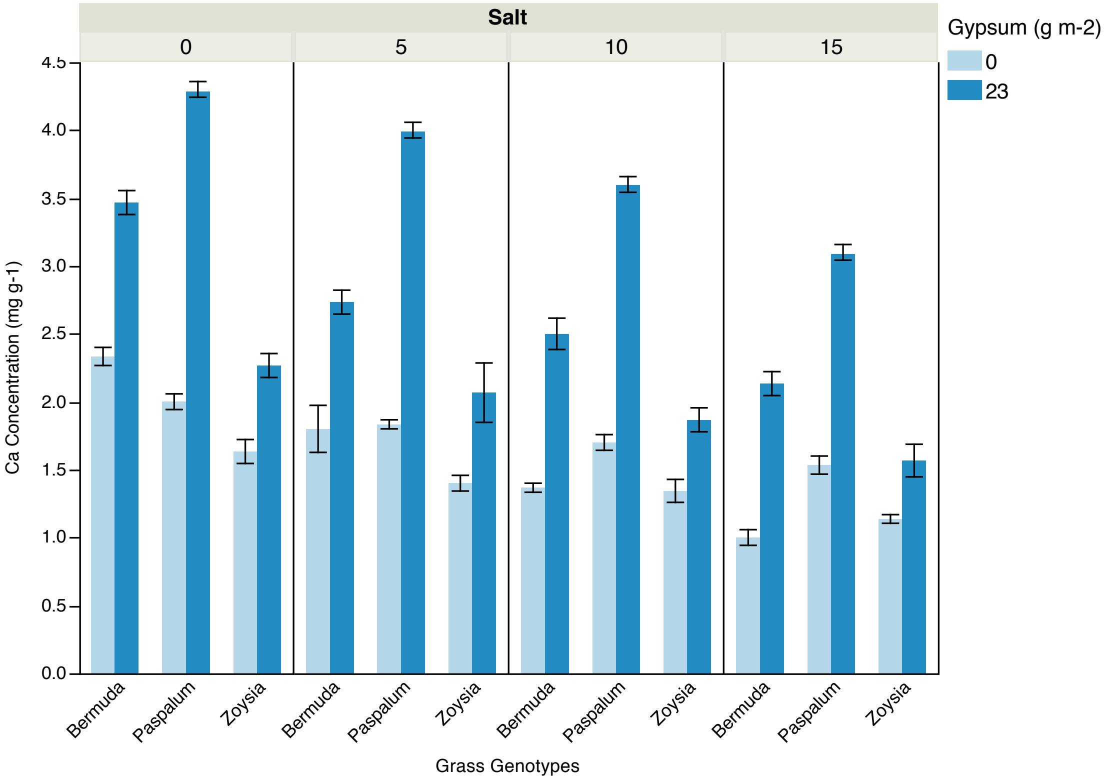


Figure B-12. Effect of salinity and gypsum on Calcium (Ca) content in root tissue of three warm season turfgrasses. Means were separated at P≤0.05 by protected LSD.



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